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According to the Pre Publication Rules, every patent application received by the United States Patent and Trademark Office after November 29, 2000 will be pre-published at eighteen months from the effective filing date. When the application is published the contents, including the sequences, will become prior art.

Two new databases have been created to hold the pre-published sequences:

**Published\_Applications\_NA** contains nucleic acid sequences; the search results will have the extension **.rnpb**.

**Published\_Applications\_AA** contains amino acid sequences; the search results will have the extension **.rapb**.

Each pre-published application is given a unique Publication Number. An example of a Publication Number is US20021234567A1. The "US" indicates the application was a U.S. application. The first 4 digits show the calendar year the application was published. The next 7 digits represent when the application was published. This 7-digit number starts at zero at the beginning of each calendar year. Each application published is given the next number in order. The "A" indicates a utility patent application and the "1" shows that this was the first time the application had been published. If the applicants submit changes to the application, they may request that the changed application be published again. In such instances, the "1" at the end of the number would be replaced by a "2".

**Sequences in the PGPub database are public information; it is permissible to leave these results in the case.**

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Dear Examiner,

The attached search was run with the most recently released version of Compugen's search software, GenCore 5. With this update, several changes have occurred in the results of FrameSearches (protein query sequence vs nucleic acid databases or nucleic acid query sequence vs protein databases).

**In reference to FrameSearches:**

- The output format has been improved so that it more closely resembles the format for standard search output.
- Calculation of Percent Similarity has been changed for FrameSearches. The new method of calculation is more similar to the method used in NCBI's BLAST algorithm. The same results are found in the same order using GenCore 5 and the previous version of GenCore, but Percent Similarities are lower in GenCore 5 results.
- The formula for % similarity calculation is:

$$100 * \frac{\text{matches} + \text{conservative substitutions}}{\text{alignment\_length}}$$

where "matches" is the number of identical matches and "conservative substitutions" is the number of non-identical positive matches.

- GenCore 4.5 considers the match Thr vs GCT (Ala) to be a similarity since BLOSUM62 gives score of 0 to this match. It is marked by ':::' in the alignment:

```
Qy      46 AspSerThrAspAla.Met..Gly 52
          |||||:::  ||| ::: |||
Db      605 GATTCGCTGCTGCTAATTTTGGC 628
```

GenCore 5 requires a positive score to consider a non-identical match a similarity, therefore the same 'match' is not emphasized in the new alignment:

```
Qy      46 AspSerThrAspAla.Met..Gly 52
          |||||  ||| ::: |||
Db      605 GATTCGCTGCTGCTAATTTTGGC 628
```

If you have any questions, please feel free to contact one of the searchers in Biotech/Chem Library.

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STIC-ILL

From: Portner, Ginny  
Sent: Thursday, January 02, 2003 4:14 PM  
To: STIC-ILL  
Subject: FW: 09/727,892  
Importance: High

NO

426283

Journal of Dairy Science, Oct. 1998, Vol. 81(10), pages 2771-2778. Bower, CK et al

9812283

*Ginny Portner*  
CM1, Art Unit 1645  
Room 7e13  
Mail box 7e12  
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STIC-ILL

Op501.C5  
Adonis

From: Portner, Ginny  
Sent: Thursday, January 02, 2003 11:35 AM  
To: STIC-ILL  
Subject: 09/727,892 (adonis reference)

Importance: High

TITLE: Bioluminescence-based assays for detection and characterization of  
bacteria and chemicals in clinical laboratories  
AUTHOR(S): Billard P; DuBow MS (REPRINT)  
CORPORATE SOURCE: UNIV METZ,CTR ENVIRONM SCI, 1 RUE RECOLLETS, BP  
94025/F-57040 METZ//FRANCE/ (REPRINT); UNIV METZ,CTR ENVIRONM SCI/F-57040  
METZ//FRANCE/  
PUBLICATION TYPE: JOURNAL  
PUBLICATION: CLINICAL BIOCHEMISTRY, 1998, V31, N1 (FEB), P1-14  
GENUINE ARTICLE#-ZE635  
PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE,  
KIDLINGTON, OXFORD, ENGLAND OX5 1GB  
ISSN: 0009-9120  
CURRENT CONTENTS JOURNAL ANNOUNCEMENT: CC LIFE, V41, N17  
LANGUAGE: English DOCUMENT TYPE: REVIEW  
GEOGRAPHIC LOCATION: FRANCE  
SUBFILE: SciSearch; CC LIFE--Current Contents/Life Sciences  
JOURNAL SUBJECT CATEGORY: MEDICAL RESEARCH, DIAGNOSIS & TREATMENT

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STIC-ILL

9184165

V NO

From: Portner, Ginny  
Sent: Thursday, January 02, 2003 2:06 PM  
To: STIC-ILL  
Subject: 09/727,892

426180

Importance: High

02583615 BIOSIS NO.: 000017031673  
EARLY FUNCTION OF A VIRULENT STAPHYLOCOCCAL PHAGE  
AUTHOR: LATHAM J M; HARRIS E F  
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL (79). 1979 281 1979  
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society  
for Microbiology  
CODEN: ASMAC  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
DESCRIPTORS: ABSTRACT PHAGE 44A- HJD STAPHYLOCOCCUS - AUREUS COVALENTLY  
CLOSED CIRCULAR DNA

0094-8519

01074744 BIOSIS NO.: 000009054954  
EFFECTS OF INCREASED SERINE CONCENTRATION IN THE CELL WALL OF  
STAPHYLOCOCCUS - AUREUS 44A HJD  
AUTHOR: DONEGAN E A; RIGGS H G JR  
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL 73. 1973 182 1973  
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society  
for Microbiology  
CODEN: ASMAC  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
DESCRIPTORS: ABSTRACT

COMPLETED

Scientific and Technical  
Information Center

JAN 06 RECD

00633656 BIOSIS NO.: 000007083621  
BACTERIO PHAGE REPRODUCTION IN LYSOSTAPHIN TREATED STAPHYLOCOCCUS - AUREUS  
44-A- HJD  
AUTHOR: ONDERDONK A B; RIGGS H G JR  
JOURNAL: BACTERIOL PROC 71. 1971 185 1971  
FULL JOURNAL NAME: Bacteriological Proceedings  
CODEN: BACPA  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
DESCRIPTORS: ABSTRACT PROTOPLAST ANTI INFECT-DRUG ELECTRON MICROSCOPE  
PHOSPHORUS-32 DNA  
CONCEPT CODES:

PAT. & T.M. OFFICE

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STIC-ILL

9184149

VNO

426187

From: Portner, Ginny  
Sent: Thursday, January 02, 2003 2:06 PM  
To: STIC-ILL  
Subject: 09/727,892

Importance: High

02583615 BIOSIS NO.: 000017031673  
EARLY FUNCTION OF A VIRULENT STAPHYLOCOCCAL PHAGE  
AUTHOR: LATHAM J M; HARRIS E F  
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL (79). 1979 281 1979  
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society  
for Microbiology  
CODEN: ASMAC  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
DESCRIPTORS: ABSTRACT PHAGE 44A- HJD STAPHYLOCOCCUS - AUREUS COVALENTLY  
CLOSED CIRCULAR DNA

01074744 BIOSIS NO.: 000009054954  
EFFECTS OF INCREASED SERINE CONCENTRATION IN THE CELL WALL OF  
STAPHYLOCOCCUS - AUREUS 44A HJD  
AUTHOR: DONEGAN E A; RIGGS H G JR  
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL 73. 1973 182 1973  
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society  
for Microbiology  
CODEN: ASMAC  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
DESCRIPTORS: ABSTRACT

0094-8519

Scientific and Technical  
Information Center

JAN 06 RECD

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00633656 BIOSIS NO.: 000007083621  
BACTERIO PHAGE REPRODUCTION IN LYSOSTAPHIN TREATED STAPHYLOCOCCUS AUREUS  
44-A- HJD  
AUTHOR: ONDERDONK A B; RIGGS H G JR  
JOURNAL: BACTERIOL PROC 71. 1971 185 1971  
FULL JOURNAL NAME: Bacteriological Proceedings  
CODEN: BACPA  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
DESCRIPTORS: ABSTRACT PROTOPLAST ANTI INFECT-DRUG ELECTRON MICROSCOPE  
PHOSPHORUS-32 DNA  
CONCEPT CODES:

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N10

From: Portner, Ginny  
Sent: Thursday, January 02, 2003 3:03 PM  
To: STIC-ILL  
Subject: FW: 09/727,892

426277

Importance: High

A Book: Methods in Molecular Biology; Drug-DNA interaction protocols  
Author: Dooley, Thomas P et al  
Editor: Fox, KR: Ed  
Journal: Methods in Molecular Microbiology, 90, page 117-12, 1997  
**ISSN:0097-0816**

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**COMPLETED**



**Inhibitors of DNA polymerase III as novel antimicrobial agents against gram-positive eubacteria.**

AUTHOR: Tarantino Paul M Jr; Zhi Chengxin; Wright George E; Brown Neal C(a)

AUTHOR ADDRESS: (a)Dept. of Pharmacology and Molecular Toxicology,  
University of Massachusetts Medical School, Worc\*\*USA

JOURNAL: Antimicrobial Agents and Chemotherapy 43 (8):p1982-1987 Aug.,  
1999

ISSN: 0066-4804

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: 6-Anilinouracils are selective inhibitors of DNA polymerase III, the enzyme required for the replication of chromosomal DNA in gram-positive bacteria (N. C. Brown, L. W. Dudycz, and G. E. Wright, Drugs Exp. Clin. Res. 12:555-564, 1986). A new class of 6-anilinouracils based on N-3 alkyl substitution of the uracil ring was synthesized and analyzed for activity as inhibitors of the gram-positive bacterial DNA polymerase III and the growth of gram-positive bacterial pathogens. Favorable in vitro properties of N-3-alkyl derivatives prompted the synthesis of derivatives in which the R group at N-3 was replaced with more-hydrophilic methoxyalkyl and hydroxyalkyl groups. These hydroxyalkyl and methoxyalkyl derivatives displayed Ki values in the range from 0.4 to 2.8  $\mu$ M against relevant gram-positive bacterial DNA polymerase IIIs and antimicrobial activity with MICs in the range from 0.5 to 15  $\mu$ g/ml against a broad spectrum of gram-positive bacteria, including methicillin-resistant staphylococci and vancomycin-resistant enterococci. Two of these hydrophilic derivatives displayed protective activity in a simple mouse model of lethal staphylococcal infection.

REGISTRY NUMBERS: 7269-15-0: 6-ANILINOURACILS; 37217-33-7: DNA POLYMERASE

09/727892

1/9/12 (Item 6 from file: 440)  
DIALOG(R) File 440:Current Contents Search(R)  
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09346349 References: 72

**TITLE: Bioluminescence-based assays for detection and characterization of bacteria and chemicals in clinical laboratories**

**AUTHOR(S):** Billard P; DuBow MS (REPRINT)

**CORPORATE SOURCE:** UNIV METZ,CTR ENVIRONM SCI, 1 RUE RECOLLETS, BP 94025/F-57040 METZ//FRANCE/ (REPRINT); UNIV METZ,CTR ENVIRONM SCI/F-57040 METZ//FRANCE/

**PUBLICATION TYPE:** JOURNAL

**PUBLICATION:** CLINICAL BIOCHEMISTRY, 1998, V31, N1 (FEB), P1-14

**GENUINE ARTICLE#:** ZE635

**PUBLISHER:** PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB

**ISSN:** 0009-9120

**CURRENT CONTENTS JOURNAL ANNOUNCEMENT:** CC LIFE, V41, N17

**LANGUAGE:** English **DOCUMENT TYPE:** REVIEW

**GEOGRAPHIC LOCATION:** FRANCE

**SUBFILE:** SciSearch; CC LIFE--Current Contents/Life Sciences

**JOURNAL SUBJECT CATEGORY:** MEDICAL RESEARCH, DIAGNOSIS & TREATMENT

**ABSTRACT:** Objectives: To survey recent advances in the application of bioluminescence to public health problems. The usefulness of bacterial (lux) and eucaryotic (luc) luciferase genes is presented, along with several examples that demonstrate their value as ''reporters'' of many endpoints of clinical concern.

*Cited*

**Conclusions:** The development of new technologies for monitoring biological and chemical contaminants is in continuous progress. Recent excitement in this area has come from the use of genes encoding enzymes for bioluminescence as reporter systems. Applications of the recombinant luciferase reporter **phage** concept now provide a sensitive approach for bacterial detection, their viability, and sensitivity to antimicrobial agents. Moreover, a number of fusions of the lux and luc genes to stress inducible genes in different bacteria can allow a real-time measurement of gene expression and determination of cellular viability, and also constitute a new tool to detect toxic chemicals and their bioavailability.

**DESCRIPTORS--Author Keywords:** bioluminescence ; luciferase ; biosensor ; reporter ; **phages** ; bacterial pathogens ; chemicals ; detection

**IDENTIFIERS--KeyWord Plus:** GREEN-FLUORESCENT PROTEIN; CHROMOSOMAL ARS

OPERON; ESCHERICHIA-COLI; FIREFLY LUCIFERASE; GENE FUSIONS;

**STAPHYLOCOCCUS - AUREUS** ; SALMONELLA-TYPHIMURIUM; TRANSIENT TRANSFECTION; SENSITIVE DETECTION; DIFFERENT COLORS

**In vitro incorporation of serine into the staphylococcal cell wall**

Donegan E.A.; Riggs Jr H.G.

Dept. Microbiol., Univ. Missouri Sch. Med., Columbia, Mo. 65201 United States

Infection and Immunity ( INFECT. IMMUN. ) 1974, 10/1 (264-269)

CODEN: INFIB

DOCUMENT TYPE: Journal

LANGUAGE: ENGLISH

A variant of *S. aureus* 44A HJD was isolated by serial growth in Trypticase soy broth to which 2 M serine had been added (wt/vol). Amino acid analysis of hydrolysates of purified mucopeptides from the variant showed that they contained 1.266 serine and 2.156 glycine residues per glutamic acid residue, compared with 0.174 serine and 3.144 glycine residues per glutamic acid residue in the mucopeptide of the parent strain. In addition to this alteration in the chemical composition of the mucopeptide, the variant lost many of the biochemical and cultural characteristics of the parent organism. The variant was not sensitive to the lytic action of lysostaphin and was non phage typable. Moreover, in vitro tests indicated that the organism was coagulase negative, did not produce gelatinase or deoxyribonuclease, and did not hemolyze sheep erythrocytes. Apparently due to the change in the serine content in the cell wall of the parent *S. aureus* strain, the organism had become 'epidermidis like' in its properties.

11232799 BIOSIS NO.: 199800014131

**DNA polymerase inhibition assay (PIA) for the detection of drug-DNA interactions.**

**BOOK TITLE: Methods in Molecular Biology; Drug-DNA interaction protocols**

**AUTHOR:** Dooley Thomas P(a); Weiland Katherine L

**BOOK AUTHOR/EDITOR:** Fox K R: Ed

**AUTHOR ADDRESS:** (a)Molecular Pharmacol., Southern Res. Inst., Birmingham, AL\*\*USA

**JOURNAL:** Methods in Molecular Biology 90p117-125 1997

**BOOK PUBLISHER:** Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA

**ISSN:** 0097-0816 **ISBN:** 0-89603-447-X

**DOCUMENT TYPE:** Book

**RECORD TYPE:** Citation

**LANGUAGE:** English

**REGISTRY NUMBERS:** 9012-90-2: DNA POLYMERASE

**DESCRIPTORS:**

**MAJOR CONCEPTS:** Methods and Techniques; Molecular Genetics (Biochemistry and Molecular Biophysics); Pharmacology

**CHEMICALS & BIOCHEMICALS:** drug; DNA--drug interaction

**METHODS & EQUIPMENT:** **DNA polymerase inhibition assay** --analytical method

**MISCELLANEOUS TERMS:** Book Chapter

**CONCEPT CODES:**

10050 Biochemical Methods-General

03502 Genetics and Cytogenetics-General

10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines

10060 Biochemical Studies-General

10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines

10804 Enzymes-Methods

09346349 References: 72

**TITLE: Bioluminescence-based assays for detection and characterization of bacteria and chemicals in clinical laboratories**

**AUTHOR(S):** Billard P; **DuBow MS (REPRINT)**

**CORPORATE SOURCE:** UNIV METZ,CTR ENVIRONM SCI, 1 RUE RECOLLETS, BP 94025/F-57040 METZ//FRANCE/ (REPRINT); UNIV METZ,CTR ENVIRONM SCI/F-57040 METZ//FRANCE/

**PUBLICATION TYPE:** JOURNAL

**PUBLICATION:** CLINICAL BIOCHEMISTRY, 1998, V31, N1 (FEB), P1-14

**GENUINE ARTICLE#:** ZE635

**PUBLISHER:** PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB

**ISSN:** 0009-9120

**CURRENT CONTENTS JOURNAL ANNOUNCEMENT:** CC LIFE, V41, N17

**LANGUAGE:** English **DOCUMENT TYPE:** REVIEW

**GEOGRAPHIC LOCATION:** FRANCE

**SUBFILE:** SciSearch; CC LIFE--Current Contents/Life Sciences

**JOURNAL SUBJECT CATEGORY:** MEDICAL RESEARCH, DIAGNOSIS & TREATMENT

**ABSTRACT:** Objectives: To survey recent advances in the application of bioluminescence to public health problems. The usefulness of bacterial (lux) and eucaryotic (luc) luciferase genes is presented, along with several examples that demonstrate their value as ''reporters'' of many endpoints of clinical concern.

**Conclusions:** The development of new technologies for monitoring biological and chemical contaminants is in continuous progress. Recent excitement in this area has come from the use of genes encoding enzymes for bioluminescence as reporter systems. Applications of the recombinant luciferase reporter **phage** concept now provide a sensitive approach for bacterial detection, their viability, and sensitivity to antimicrobial agents. Moreover, a number of fusions of the lux and luc genes to stress inducible genes in different bacteria can allow a real-time measurement of gene expression and determination of cellular viability, and also constitute a new tool to detect toxic chemicals and their bioavailability.

**DESCRIPTORS--Author Keywords:** bioluminescence ; luciferase ; biosensor ; reporter ; **phages** ; bacterial pathogens ; chemicals ; detection

**IDENTIFIERS--KeyWord Plus:** GREEN-FLUORESCENT PROTEIN; CHROMOSOMAL ARS

OPERON; ESCHERICHIA-COLI; FIREFLY LUCIFERASE; GENE FUSIONS;

**STAPHYLOCOCCUS - AUREUS** ; SALMONELLA-TYPHIMURIUM; TRANSIENT TRANSFECTION; SENSITIVE DETECTION; DIFFERENT COLORS

3/9/15 (Item 15 from file: 156)

DIALOG(R) File 156:ToxFile

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01259182 98297420 PMID: 9635497

**An ergosterol peroxide, a natural product that selectively enhances the inhibitory effect of linoleic acid on DNA polymerase beta.**

Mizushina Y; Watanabe I; Togashi H; Hanashima L; Takemura M; Ohta K; Sugawara F; Koshino H; Esumi Y; Uzawa J; Matsukage A; Yoshida S; Sakaguchi K

Department of Applied Biological Science, Science University of Tokyo, Chiba, Japan.

Biological & pharmaceutical bulletin (JAPAN) May 1998, 21 (5) p444-8

, ISSN 0918-6158 Journal Code: 9311984

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: Toxbib ; INDEX MEDICUS

As described previously (Mizushina Y., Tanaka N., Yagi H., Kurosawa T., Onoue M., Seto H., Horie T., Aoyagi N., Yamaoka M., Matsukage A., Yoshida S., and Sakaguchi K., Biochim. Biophys. Acta, 1308, 256-262, 1996), linoleic acid (LA) inhibits the activities of mammalian DNA polymerases. We found a natural product from a basidiomycete, Ganoderma lucidum, that enhances this effect of LA in a special manner. The structure was identified to be an ergosterol peroxide, 5,8-epidioxy-5alpha,8alpha-ergosta-6,22E-dien-3beta-ol by spectroscopic analyses. The ergosterol peroxide (EPO) itself scarcely inhibited the activities of calf thymus DNA polymerase alpha (pol. alpha) or rat DNA polymerase beta (pol. beta). However, when EPO at 0.25 mM was present, 10 microM or less of LA almost completely inhibited the pol. beta activity, while almost complete inhibition by LA itself was achieved at 80 microM or higher. Interestingly, under the same conditions, EPO did not affect the LA-effect on pol. alpha. The action mode of the EPO was discussed.

Tags: Animal; Support, Non-U.S. Gov't

Descriptors: DNA Polymerase beta --antagonists and inhibitors--AI; \*Enzyme Inhibitors--pharmacology--PD; \*Ergosterol--analogs and derivatives --AA; \*Linoleic Acid--pharmacology--PD; Basidiomycota--enzymology--EN; Cattle; Chromatography, High Pressure Liquid; DNA Polymerase I--antagonists and inhibitors--AI; Drug Synergism; Ergosterol--pharmacology--PD; Fatty Acids--chemistry--CH; Fatty Acids--metabolism--ME; Rats

CAS Registry No.: 0 (Enzyme Inhibitors); 0 (Fatty Acids); 2061-64-5 (ergosterol-5,8-peroxide); 2197-37-7 (Linoleic Acid); 57-87-4 (Ergosterol)

Enzyme No.: EC 2.7.7.- (DNA Polymerase I); EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19980824

3/9/16 (Item 16 from file: 156)

DIALOG(R) File 156:ToxFile

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01252163 98250009 PMID: 9590127

**Sulfated glycosylglycerolipid from archaebacterium inhibits eukaryotic DNA polymerase alpha, beta and retroviral reverse transcriptase and affects methyl methanesulfonate cytotoxicity.**

Ogawa A; Murate T; Izuta S; Takemura M; Furuta K; Kobayashi J; Kamikawa T; Nimura Y; Yoshida S

First Department of Surgery, Nagoya University School of Medicine, Japan.

International journal of cancer. Journal international du cancer (UNITED STATES) May 18 1998, 76 (4) p512-8, ISSN 0020-7136 Journal Code: 0042124

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: Toxbib ; INDEX MEDICUS; AIDS/HIV

A sulfated glycosylglycerolipid, 1-O-(6'-sulfo-alpha-D-glucopyranosyl)-2,3-d

i-O-phytanyl- sn-glycerol (KN-208), a derivative of the polar lipid isolated from an archaeobacterium, strongly inhibited DNA polymerase (pol) alpha and pol beta in vitro among 5 eukaryotic DNA polymerases (alpha, beta, gamma, delta, and epsilon). It also inhibited Escherichia coli DNA polymerase I Klenow fragment (E. coli pol I) and human immunodeficiency virus reverse transcriptase (HIV RT). The mode of inhibition of these polymerases was competitive with the DNA template primer and was non-competitive with the substrate dTTP. KN-208 inhibited pol beta most strongly, with a  $K_i$  value of 0.05 microM, 10-fold lower than that for pol alpha (0.5 microM) and 60- or 140-fold lower than that for HIV RT (3 microM) or for E. coli pol I (7 microM), respectively. The loss of sulfate on the 6'-position of glucopyranoside of this compound completely abrogated inhibition. However, the hydrophilic part of KN-208, glucose 6-sulfate alone, showed no inhibition. Other sulfated compounds containing different hydrophobic structures, such as dodecyl sulfate and cholesterol sulfate, exhibited a much weaker inhibition. Our results suggest that the whole molecular structure of KN-208 is required for inhibition. KN-208 was shown to be modestly cytotoxic for the human leukemic cell line K562. Interestingly, a subcytotoxic dose of KN-208 increased the sensitivity of the human leukemic cells to an alkylating agent, methyl methanesulfonate, while it did not potentiate the effects of ultraviolet light or of cisplatin.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: \*Archaea--enzymology--EN; \*DNA-Directed DNA Polymerase --antagonists and inhibitors--AI; \*Glycolipids--pharmacology--PD; \*HIV --enzymology--EN; \*Methyl Methanesulfonate--pharmacology--PD; \*Reverse Transcriptase Inhibitors; Cell Division; DNA Polymerase I--antagonists and inhibitors--AI; **DNA Polymerase beta** --antagonists and inhibitors--AI; Glycolipids--chemistry--CH; Leukemia, Experimental; Tumor Cells, Cultured  
CAS Registry No.: 0 (1-O-(6'-sulfo-alpha-D-glucopyranosyl)-2,3-di-O-phytanyl-sn-glycerol); 0 (Glycolipids); 0 (Reverse Transcriptase Inhibitors); 66-27-3 (Methyl Methanesulfonate)

Enzyme No.: EC 2.7.7.- (DNA Polymerase I); EC 2.7.7.- (DNA Polymerase beta); EC 2.7.7.7 (DNA-Directed DNA Polymerase)

Record Date Created: 19980604

?t s13/9/23 26 46

13/9/23 (Item 23 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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12109422 BIOSIS NO.: 199900404271

**Inhibitors of DNA polymerase III as novel antimicrobial agents against gram-positive eubacteria.**

AUTHOR: Tarantino Paul M Jr; Zhi Chengxin; Wright George E; Brown Neal C(a)

AUTHOR ADDRESS: (a)Dept. of Pharmacology and Molecular Toxicology,

University of Massachusetts Medical School, Worc\*\*USA

JOURNAL: Antimicrobial Agents and Chemotherapy 43 (8):p1982-1987 Aug., 1999

ISSN: 0066-4804

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

**ABSTRACT:** 6-Anilinouracils are selective inhibitors of DNA polymerase III, the enzyme required for the replication of chromosomal DNA in gram-positive bacteria (N. C. Brown, L. W. Dudycz, and G. E. Wright, Drugs Exp. Clin. Res. 12:555-564, 1986). A new class of 6-anilinouracils based on N-3 alkyl substitution of the uracil ring was synthesized and analyzed for activity as inhibitors of the gram-positive bacterial DNA polymerase III and the growth of gram-positive bacterial pathogens. Favorable in vitro properties of N-3-alkyl derivatives prompted the synthesis of derivatives in which the R group at N-3 was replaced with more-hydrophilic methoxyalkyl and hydroxyalkyl groups. These hydroxyalkyl and methoxyalkyl derivatives displayed  $K_i$  values in the range from 0.4 to 2.8  $\mu$ M against relevant gram-positive bacterial DNA polymerase IIIs and antimicrobial activity with MICs in the range from 0.5 to 15  $\mu$ g/ml against a broad spectrum of gram-positive bacteria, including

methicillin-resistant staphylococci and vancomycin-resistant enterococci. Two of these hydrophilic derivatives displayed protective activity in a simple mouse model of lethal staphylococcal infection.

REGISTRY NUMBERS: 7269-15-0: 6-ANILINOURACILS; 37217-33-7: DNA POLYMERASE III

DESCRIPTORS:

MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics); Infection; Pharmacology

BIOSYSTEMATIC NAMES: Gram-Positive Cocci--Eubacteria, Bacteria, Microorganisms; Micrococcaceae--Gram-Positive Cocci, Eubacteria, Bacteria, Microorganisms; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia

ORGANISMS: enterococci (Gram-Positive Cocci)--pathogen, vancomycin-resistant; mouse (Muridae)--model; staphylococci (Micrococcaceae)--methicillin-resistant, pathogen

BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates

DISEASES: staphylococcal infection--bacterial disease

CHEMICALS & BIOCHEMICALS: DNA polymerase III; 6-anilinouracils-- DNA polymerase III inhibitor , antimicrobial activity

CONCEPT CODES:

38504 Chemotherapy-Antibacterial Agents  
10060 Biochemical Studies-General  
10802 Enzymes-General and Comparative Studies; Coenzymes  
12512 Pathology, General and Miscellaneous-Therapy (1971- )  
13002 Metabolism-General Metabolism; Metabolic Pathways  
36002 Medical and Clinical Microbiology-Bacteriology

BIOSYSTEMATIC CODES:

07700 Gram-Positive Cocci (1992- )  
07702 Micrococcaceae (1992- )  
86375 Muridae

13/9/26 (Item 26 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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11898957 BIOSIS NO.: 199900145066

**Nucleic acid ligand inhibitors to DNA polymerases.**

AUTHOR: Gold L; Jayasena S D

AUTHOR ADDRESS: Boulder, Colo.\*\*USA

JOURNAL: Official Gazette of the United States Patent and Trademark Office  
Patents 1219 (4):p3394 Feb. 23, 1999

PATENT NUMBER: US 5874557 PATENT DATE GRANTED: Feb. 23, 1999 19990223

PATENT ASSIGNEE: NEXSTAR PHARMACEUTICALS, INC. PATENT COUNTRY: USA

ISSN: 0098-1133

RECORD TYPE: Citation

LANGUAGE: English

MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics); Pharmacology

MISCELLANEOUS TERMS: Patent; BIOTECHNOLOGY; DNA POLYMERASE INHIBITOR ; ENZYME INHIBITOR; NUCLEIC ACID LIGAND; PHARMACEUTICALS

13/9/46 (Item 46 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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10453687 BIOSIS NO.: 199699074832

**An application of MTT-colorimetric assay for the screening of anti-adenovirus agents.**

AUTHOR: Kodama E(a); Shigeta S; Suzuki T; De Clercq E

AUTHOR ADDRESS: (a)Dep. Pediatr., Univ. Alabama at Birmingham, AL\*\*USA

JOURNAL: Antiviral Research 30 (1):pA54 1996

CONFERENCE/MEETING: Ninth International Conference on Antiviral Research  
Urabandai, Japan May 19-24, 1996

ISSN: 0166-3542



RECORD TYPE: Citation

LANGUAGE: English

REGISTRY NUMBERS: 9012-90-2: DNA POLYMERASE

DESCRIPTORS:

MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology;  
Infection; Methods and Techniques; Pathology; Pharmacology

BIOSYSTEMATIC NAMES: Adenoviridae--Viruses; Hominidae--Primates, Mammalia  
, Vertebrata, Chordata, Animalia

ORGANISMS: Adenoviridae (Adenoviridae); Hominidae (Hominidae)

BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): animals; chordates; humans;  
mammals; microorganisms; primates; vertebrates; viruses

CHEMICALS & BIOCHEMICALS: DNA POLYMERASE

MISCELLANEOUS TERMS: ANTIVIRAL-DRUG; **DNA POLYMERASE INHIBITOR** ; HUMAN

MKN-28 CELLS; MEETING ABSTRACT; MEETING POSTER; SCREENING METHOD

CONCEPT CODES:

10010 Comparative Biochemistry, General  
10504 Biophysics-General Biophysical Techniques  
10506 Biophysics-Molecular Properties and Macromolecules  
12512 Pathology, General and Miscellaneous-Therapy (1971- )  
22002 Pharmacology-General  
22003 Pharmacology-Drug Metabolism; Metabolic Stimulators  
32600 In Vitro Studies, Cellular and Subcellular  
36006 Medical and Clinical Microbiology-Virology  
38506 Chemotherapy-Antiviral Agents  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals  
10060 Biochemical Studies-General  
10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines  
10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
10808 Enzymes-Physiological Studies  
13012 Metabolism-Proteins, Peptides and Amino Acids  
13014 Metabolism-Nucleic Acids, Purines and Pyrimidines  
14001 Digestive System-General; Methods  
24005 Neoplasms and Neoplastic Agents-Neoplastic Cell Lines  
32500 Tissue Culture, Apparatus, Methods and Media  
33506 Virology-Animal Host Viruses

BIOSYSTEMATIC CODES:

02601 Adenoviridae (1993- )  
86215 Hominidae

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06jan03 11:04:17 User228206 Session D1900.4

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 \$0.21      TELNET  
 \$8.57      Estimated cost this search  
 \$8.57      Estimated total session cost    0.373 DialUnits

### Status: Signed Off. (1 minutes)

10389336 99353975 PMID: 10423540

**The cyanogenic glucoside, prunasin (D-mandelonitrile-beta-D-glucoside), is a novel inhibitor of DNA polymerase beta.**

Mizushima Y; Takahashi N; Ogawa A; Tsurugaya K; Koshino H; Takemura M; Yoshida S; Matsukage A; Sugawara F; Sakaguchi K

Department of Applied Biological Science, Science University of Tokyo, Noda, Chiba, 278-8510, Japan.

Journal of biochemistry (JAPAN) Aug 1999, 126 (2) p430-6, ISSN 0021-924X Journal Code: 0376600

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A DNA polymerase beta (pol. beta) inhibitor has been isolated independently from two organisms; a red perilla, *Perilla frutescens*, and a mugwort, *Artemisia vulgaris*. These molecules were determined by spectroscopic analyses to be the cyanogenic glucoside, D-mandelonitrile-beta-D-glucoside, prunasin. The compound inhibited the activity of rat pol. beta at 150 microM, but did not influence the activities of calf DNA polymerase alpha and plant DNA polymerases, human immunodeficiency virus type 1 reverse transcriptase, calf terminal deoxynucleotidyl transferase, or any prokaryotic DNA polymerases, or DNA and RNA metabolic enzymes examined. The compound dose-dependently inhibited pol. beta activity, the IC(50) value being 98 microM with poly dA/oligo dT(12-18) and dTTP as the DNA template and substrate, respectively. Inhibition of pol. beta by the compound was competitive with the substrate, dTTP. The inhibition was enhanced in the presence of fatty acid, and the IC(50) value decreased to approximately 40 microM. In the presence of C(10)-decanoic acid, the K(i) value for substrate dTTP decreased by 28-fold, suggesting that the fatty acid allowed easier access of the compound to the substrate-binding site.

Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't

Descriptors: **DNA Polymerase beta** --antagonists and inhibitors--AI; \*Enzyme Inhibitors--chemistry--CH; \*Enzyme Inhibitors--pharmacokinetics--PK; \*Nitriles--chemistry--CH; \*Nitriles--pharmacokinetics--PK; Amygdalin--chemistry--CH; Amygdalin--pharmacokinetics--PK; *Artemisia*--chemistry--CH; *Artemisia*--enzymology--EN; Cattle; Decanoic Acids--pharmacology--PD; Dose-Response Relationship, Drug; Enzyme Inhibitors --isolation and purification--IP; Inhibitory Concentration 50; Kinetics; Lamiaceae --chemistry--CH; Nitriles--isolation and purification--IP; Plants, Medicinal; Rats; Thymine Nucleotides--chemistry--CH

CAS Registry No.: 0 (Decanoic Acids); 0 (Enzyme Inhibitors); 0 (Nitriles); 0 (Thymine Nucleotides); 138-53-4 (prunasin); 29883-15-6 (Amygdalin); 334-48-5 (decanoic acid); 611-60-9 (2',3'-dideoxythymidine triphosphate)

Enzyme No.: EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19991213

10387061 99355950 PMID: 10425125

**Harbinatic acid, a novel and potent DNA polymerase beta inhibitor from *Hardwickia binata*.**

Deng J Z; Starck S R; Hecht S M; Ijames C F; Hemling M E  
Departments of Chemistry and Biology, University of Virginia,  
Charlottesville, Virginia 22901, USA.

Journal of natural products (UNITED STATES) Jul 1999, 62 (7) p1000-2  
, ISSN 0163-3864 Journal Code: 7906882

Contract/Grant No.: CA50771; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Bioassay-guided fractionation of an active methyl ethyl ketone extract of *Hardwickia binata*, using an assay sensitive to DNA polymerase beta inhibition, resulted in the isolation of a potent inhibitor. This proved to be a novel diterpenoid, which has been named harbinatic acid (1). The structure of 1 was established as 3alpha-O-trans-p-coumaroyl-7-labden-15-oi c acid from spectroscopic analysis and by comparison with the published data for a structurally related compound. Compound 1 strongly inhibited calf thymus DNA polymerase beta, with an IC(50) value of 2.9 &mgr;M.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: **DNA Polymerase beta** --antagonists and inhibitors--AI;  
\*Diterpenes--isolation and purification--IP; \*Enzyme Inhibitors--isolation  
and purification--IP; \*Fabaceae--chemistry--CH; \*Plants, Medicinal;  
Diterpenes--pharmacology--PD; Enzyme Inhibitors--pharmacology--PD; India;  
Magnetic Resonance Spectroscopy; Plant Extracts--chemistry--CH; Plant  
Extracts--pharmacology--PD

CAS Registry No.: 0 (Diterpenes); 0 (Enzyme Inhibitors); 0 (Plant  
Extracts); 0 (harbinatic acid)

Enzyme No.: EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19991015

10233734 99197137 PMID: 10096862

**bis-5-Alkylresorcinols from Panopsis rubescens that inhibit DNA polymerase beta.**

Deng J Z; Starck S R; Hecht S M  
Department of Chemistry, University of Virginia, Charlottesville,  
Virginia 22901, USA.

Journal of natural products (UNITED STATES) Mar 1999, 62 (3) p477-80  
, ISSN 0163-3864 Journal Code: 7906882

Contract/Grant No.: CA50771; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Bioassay-guided fractionation of *Panopsis rubescens*, using an assay to detect DNA polymerase beta inhibition, led to the isolation of two new bis-5-alkylresorcinols (1 and 2), in addition to one known bis-5-alkylresorcinol (3). The structures of 1-3 were established as 1,3-dihydroxy-5-[14'-(3' ',5' '-dihydroxyphenyl)-cis-4'-tetradecenyl]benzene (1), 1,3-dihydroxy-5-[14'-(3' ',5' '-dihydroxyphenyl)-cis-7'-tetradecenyl]benzene (2), and 1,3-dihydroxy-5-[14'-(3' ',5' '-dihydroxyphenyl)tetradecenyl]benzene (3), respectively, by spectroscopic and chemical analyses. Compounds 1-3 exhibited potent inhibition of calf thymus DNA polymerase beta, with IC50 values of 7.5, 6.5, and 5.8 microM, respectively.

Tags: Animal; Support, U.S. Gov't, P.H.S.

Descriptors: **DNA Polymerase beta** --antagonists and inhibitors--AI;  
\*Enzyme Inhibitors--chemistry--CH; \*Plants, Medicinal--chemistry--CH;  
\*Resorcinols--chemistry--CH; Acetylation; Cattle; Enzyme Inhibitors  
--isolation and purification--IP; Magnetic Resonance Spectroscopy; Plant  
Extracts--chemistry--CH; Plant Stems--chemistry--CH; Resorcinols--isolation  
and purification--IP; Spectrometry, Mass, Fast Atom Bombardment

CAS Registry No.: 0 (Enzyme Inhibitors); 0 (Plant Extracts); 0  
(Resorcinols)

Enzyme No.: EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19990607

Set	Items	Description
S1	79	'DNA POLYMERASE BETA --ANTAGONISTS AND INHIBITO'
S2	1335	R1-R3
S3	1023	E1-E50
S4	1	'DNA POLYMERASE III BETA SUBUNIT'
S5	2	'DNA POLYMERASE III BETA SUBUNIT DNAN GENE' OR 'DNA POLYME- RASE III BETA-SUBUNIT'
S6	2	'DNA POLYMERASE III INHIBITOR'
S7	75	E27-E31
S8	1	'DNA POLYMERASE INHIBITION ASSAY'
S9	1	'DNA POLYMERASE POL' OR S27
S10	1	'DNA POLYMERASE STOP ASSAY'
S11	3	'DNA POLYMERASE SUBUNIT'
S12	219	E24-E41
S13	2081	(S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12)
S14	93	S13 AND (BACTERIOPHAGE? OR PHAGE?)
S15	62	RD (unique items)
S16	15869122	POL3 OR POLLLL OR (POL (2N) 111 OR III OR LLL OR 3)
S17	15870721	S13 OR S16 OR DNAN
S18	3107853	S17 AND (ANTIBACT? OR ANTIBIO? OR ANTIMICROB? OR ANTAGON? - OR BLOCK? OR INHIBIT? OR MODULAT?)
S19	96283	S17 AND (STAPH? OR AUREUS? OR SAUREUS?)
S20	3109	S19 AND (BACTERIOPHAG? OR PHAGE?)
S21	64210	S17 AND (BACTERIOPHAG? OR PHAGE?)
S22	1331	S19 AND S20 AND S18
S23	899	RD (unique items)
S24	105	S23/2000:2002
S25	794	S23 NOT S24
S26	50	TARGET - S25

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1999 HOLIN?

S27 0 S13 AND HOLIN?

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Ref	Items	Index-term
E1	2	HOLIMONT
E2	1	HOLIMUM
E3	580	*HOLIN
E4	1	HOLIN FUNCTION
E5	7	HOLIN GENE
E6	1	HOLIN GENE HOL-2438
E7	1	HOLIN GENES
E8	1	HOLIN HOL GENE
E9	1	HOLIN HOL PROTEIN
E10	1	HOLIN HOMOLOGY
E11	2	HOLIN INHIBITOR
E12	1	HOLIN INTEGRAL MEMBRANE PROTEINS

Enter P or PAGE for more

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S28 1999 HOLIN?

?s s28 and polymerase?

1999 S28

1070157 POLYMERASE?

S29 81 S28 AND POLYMERASE?

?s s29/2000:2002

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Processing

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>>> or undefined in one or more files.

>>>Year ranges not supported in one or more files

Completed processing all files

80 S29

13867868 PY=2000 : PY=2002

S30 19 S29/2000:2002

?s s29 not s30

81 S29

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          19 S30
S31      62 S29 NOT S30
?target s31/all
Your TARGET search request will retrieve up to 50 of the statistically most
relevant records.
Searching ALL records
...Processed 10   out of 27  files
...Processed 20   out of 27  files
...Processing Complete
      S32      50 TARGET - S31
Ending TARGET search. Enter TARGET to do another search in the present
file(s), or BEGIN new file(s).  Enter LOGOFF to disconnect from Dialog
?t s32/free/all
>>>"FREE" is not a valid format name in file(s): 399
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15/9/17 (Item 17 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

09674800 98101633 PMID: 9440683

**A mechanism for all polymerases.**

Steitz T A

Nature (ENGLAND) Jan 15 1998, 391 (6664) p231-2, ISSN 0028-0836

Journal Code: 0410462

Comment on Nature. 1998 Jan 15;391(6664) 251-8; Comment on PMID 9440688;

Comment on Nature. 1998 Jan 15;391(6664):304-7; Comment on PMID 9440698

Document type: Comment; News

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Descriptors: \*DNA-Directed DNA Polymerase--metabolism--ME; Bacillus  
stearothermophilus--enzymology--EN; Catalysis; Crystallography, X-Ray; DNA  
--metabolism--ME; **DNA Polymerase beta** --chemistry--CH; **DNA Polymerase  
beta** --metabolism--ME; DNA-Directed DNA Polymerase--chemistry--CH;  
Escherichia coli--metabolism--ME; Magnesium--chemistry--CH; Magnesium  
--metabolism--ME; Models, Molecular; Protein Conformation; Thioredoxin  
--metabolism--ME

CAS Registry No.: 52500-60-4 (Thioredoxin); 7439-95-4 (Magnesium);  
9007-49-2 (DNA)

Enzyme No.: EC 2.7.7.- (DNA Polymerase beta); EC 2.7.7.- (  
**bacteriophage** T7 induced DNA polymerase); EC 2.7.7.7 (DNA-Directed DNA  
Polymerase)

Record Date Created: 19980130

*Printed*



**Genetic Selection of Peptide Inhibitors of Biological Pathways**

Norman, Thea C.<CRF RID="C1"> ; Smith, Dana L.; Sorger, Peter K.; Drees, Becky L.; O'Rourke, Sean M.; Hughes, Timothy R.; Roberts, Christopher J.; Friend, Stephen H.; Fields, Stan; Murray, Andrew W.

Department of Physiology, University of California, San Francisco, CA 94143-0444, USA. Department of Genetics and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA. Department of Biochemistry, University of California, San Francisco, CA 94143-0448, USA. Rosetta Inpharmatics, Kirkland, WA 98034, USA.

Science Vol. 285 5427 pp. 591

Publication Date: 7-23-1999 (990723) Publication Year: 1999

Document Type: Journal ISSN: 0036-8075

Language: English

Section Heading: REPORTS

Word Count: 3728

**Abstract:** Genetic selections were used to find peptides that inhibit biological pathways in budding yeast. The peptides were presented inside cells as peptamers, surface loops on a highly expressed and biologically inert carrier protein, a catalytically inactive derivative of staphylococcal nuclease. Peptamers that inhibited the pheromone signaling pathway, transcriptional silencing, and the spindle checkpoint were isolated. Putative targets for the inhibitors were identified by a combination of two-hybrid analysis and genetic dissection of the target pathways. This analysis identified Ydr517w as a component of the spindle checkpoint and reinforced earlier indications that Ste50 has both positive and negative roles in pheromone signaling. Analysis of transcript arrays showed that the peptamers were highly specific in their effects, which suggests that they may be useful reagents in organisms that lack sophisticated genetics as well as for identifying components of existing biological pathways that are potential targets for drug discovery.

**Text:** Peptide-protein interactions have critical roles in biology. Many signals are transmitted by the binding of peptides to cell-surface receptors, and many protein-protein interactions inside cells are dominated by the binding of a peptide on one protein to a pocket on another. These interactions have inspired methods to select members of random peptide libraries that bind to known protein targets displayed on the outside of viruses (phage display) (B1) or within cells (B2). An alternative strategy is to select peptides whose binding to unknown targets produces a phenotype in the same way that mutations produce phenotypes by inactivating genes (B3). Like mutations, peptides can be used to probe the function and mechanism of biological pathways as well as to identify their in vivo protein targets.

We developed methods to express peptamers, peptides displayed as an exposed loop on the surface of an inert carrier protein, at high concentrations in budding yeast cells. This approach protects the peptides from proteolytic degradation and imposes some conformational rigidity (B4). A catalytically inactive version of staphylococcal nuclease (B5) was used as a carrier protein because it is small, folds spontaneously without chaperones, has a prominently exposed loop on its surface (B6), and can be strongly expressed as a soluble protein in eukaryotes and prokaryotes. The peptamer libraries contained 16 random amino acids inserted into the staphylococcal nuclease open reading frame (ORF) in place of the carrier's most exposed surface loop (B7).

Because the extent of pathway inhibition depends on inhibitor concentration, we maximized expression of the peptamers. A high-copy vector was made that contains a strong constitutive promoter driving the expression of a staphylococcal nuclease gene that uses optimal codons for efficient translation and epitope tags for immunological detection and protein purification (B8). Cells containing this vector expressed the peptamers as one of the most abundant proteins in the cell (Fig. 1).

We developed selections for inhibitors of two signal transduction pathways, the spindle checkpoint (B9) and the mating pheromone response pathway (B10). The spindle checkpoint arrests cells in mitosis in response to chromosomes that fail to attach to the mitotic spindle (B11) and the pheromone pathway arrests cells in G<sub>1</sub> in response to a peptide mating

factor. Both pathways are good targets for inhibitor selection because neither is essential for viability, and activation of either pathway prevents cell proliferation, creating a selection for peptamers that inhibit the pathway.

The spindle checkpoint is evolutionarily conserved and is defective in many human tumor cell lines (B12). Selecting for inhibitors of the spindle checkpoint requires genetic trickery. In normal cells, the checkpoint is activated by improperly aligned chromosomes, and overriding the checkpoint in these cells leads to errors in chromosome segregation and cell death (B13). However, overexpression of the checkpoint protein Mps1 activates the checkpoint in cells that have normal spindles (B14). In this situation, inactivating the checkpoint allows cells to divide and form viable colonies. Thus, we engineered the selection strain to overexpress Mps1 when grown on galactose (B15). We identified inhibitors of the spindle checkpoint by transforming the peptamer library into this strain and selecting for the rare transformants that formed colonies on galactose-containing medium (B16). From a pool of  $6.5 \times 10^8$  transformants, we identified three peptamers that allow cells to proliferate on galactose (Fig. 2A). Two of the peptamers reduced the amount of the Mps1 protein (Fig. 2B). This effect appeared to be on the folding or stability of Mps1, because neither inhibitor reduced the amount of (beta)-galactosidase or another protein kinase (Cdc28) expressed from the same promoter (B17).

We tested whether the putative checkpoint inhibitors could overcome the arrest caused by two other perturbations that activate the spindle checkpoint: depolymerization of mitotic spindle (Fig. 2C) and the presence of short linear minichromosomes (B17). Only the inhibitor that did not alter the amount of Mps1 expression overcame the mitotic arrest caused by these other perturbations, which suggests that it alone is a general inhibitor of the spindle checkpoint.

The phenotypes of peptamers likely depend on binding to a protein target. We used the two-hybrid technique, which detects protein-protein interactions in yeast, as one approach to identify possible targets of inhibition (B18). We fused the spindle checkpoint inhibitor to a DNA binding domain and tested it for interactions with a panel that expresses more than 85% of yeast genes fused to a transcriptional activation domain. In this assay, the inhibitor interacted strongly with an ORF of unknown function (YDR517W), which is the closest yeast homolog of GRASP65, a mammalian protein that is associated with the Golgi apparatus (B19). Cells lacking Ydr517w have defects in the spindle checkpoint (Fig. 2D). We believe that the peptamer that binds to Ydr517w also interacts with other proteins because the peptamer overcomes the effects of Mps1 overexpression more strongly than the deletion of Ydr517w, even when the peptamer is expressed in ydr517w (Delta) cells. Green fluorescent protein fused to the COOH-terminus of Ydr517w produces punctate cytoplasmic fluorescence. Although the role of a cytoplasmic protein in the spindle checkpoint is not immediately obvious, our analysis shows that identifying proteins that peptamers bind to can uncover additional members of well-studied pathways.

We also isolated inhibitors of the pheromone response pathway. Budding yeast exist in two mating types,  $\alpha$  and  $a$ , which can mate with each other only when both cells are in the G<sub>1</sub> phase of the cell cycle. The  $\alpha$  cells secrete a factor, which arrests  $a$  cells in G<sub>1</sub> (Fig. 3A). Two types of peptamers allow  $a$  cells to form colonies on plates containing a factor: those that interfere with pheromone signaling directly and those that interfere with transcriptional silencing (B20). The latter class cause haploid  $a$  cells to behave as pheromone-insensitive  $a/a$  diploids by allowing them to express a copy of the  $\alpha$  genes (HML $\alpha$ ) that is present in  $\alpha$  cells but is normally transcriptionally silent. Deleting HML $\alpha$  restores  $a$  factor sensitivity in strains that carry silencing inhibitors but has no effect on the phenotype of signaling inhibitors.

We isolated 29 peptamers that allowed  $a$  cells to proliferate in the presence of a factor, of which 20 are silencing inhibitors (Table 1). Selecting for cell proliferation demands a minimum specificity of peptamers, because those that strongly inhibit essential processes are not recovered. To test the specificity of the silencing inhibitors more stringently, we performed a global analysis of peptamer effects on transcription. Using whole genome DNA microarray analysis, we compared the pattern of transcription of strains expressing two of the silencing inhibitors to that of a dominant-negative SIR4 mutant (SIR4<sup>sup(DN)</sup>), which

disrupts repression at the silent mating-type loci and at telomeres. The transcriptional effects of the peptamers were highly correlated with those of SIR4.sup(DN) (Fig. 3B). Many MATa-specific genes (for example, STE2 and BAR1) and haploid-specific genes (for example, GPA1) showed correlated decreases in the abundance of their mRNAs, whereas the amount of MATa2 mRNA increased 1.5-to 2-fold, consistent with derepression of the silent loci. Furthermore, transcription of a number of genes located very near telomeres, including COS12, YER188W, and YAL069W, increased 1.6-to 2.5-fold, consistent with derepression of telomeric silencing. The absence of other widespread changes shows that the peptamers specifically inhibit silencing rather than causing general perturbations of cellular transcription.

We used the two-hybrid method to find proteins that interacted with five of the silencing inhibitors. Four of the five peptamers interacted strongly with specific proteins. One interacted with Asf1, whose overexpression or removal diminishes silencing (B21). Another peptamer bound to Sfh1, an essential chromatin remodeling factor that interacts physically with components required for nucleosome restructuring (B22).

Two silencing inhibitors that contain tryptophan-rich sequences (Table 1) interacted with two proteins, Tec1 and Dig1, and one of these peptamers also interacted weakly with Ste12. Both Tec1 and Dig1 interact with Ste12, a transcription factor required for the pheromone response; Dig1 was originally characterized as a negative regulator of Ste12 (B23), and Tec1 interacts with Ste12 to stimulate pseudohyphal growth in diploids and invasive growth in haploids (B24). Both peptamers blocked pseudohyphal growth in diploids, and one blocked invasive growth in haploids (B25), phenotypes shared by both *tec1* (Delta) and *ste12* (Delta) mutants. The ability of these peptamers to inhibit silencing does not appear to depend on their interaction with Dig1, Tec1, or Ste12 because mating type silencing is maintained in a *dig1* (Delta) *tec1* (Delta) mutant and telomeric silencing is maintained in a *ste12* mutant (B25).

To characterize the nine pheromone signaling inhibitors, we used genetic tests to dissect the signaling pathway (Table 2). All the peptamers appeared to interfere downstream of Ste4, the G protein that interacts with the pheromone receptor, and upstream of Far1 the cyclin-dependent kinase inhibitor that directly induces cell cycle arrest (B26). Five peptamers blocked a constitutively active allele of STE11(B27), a mitogen-activated protein (MAP) kinase kinase kinase, from inducing transcription of a pheromone-responsive reporter gene, suggesting that they interfere with the MAP kinase cascade. We analyzed these inhibitors in more detail. The responses to pheromones and high osmolarity share a MAP kinase kinase kinase (Ste11) and certain other components (Ste20 and Ste50) but use different MAP kinases to produce their final output (B28). A single peptamer increased the osmotic sensitivity of strains that depend on Ste11, Ste20, and Ste50 for osmotic signaling (Table 2), which suggests that this peptamer inhibits Ste11, Ste20, or Ste50. Two-hybrid experiments supported this conclusion: this peptamer interacted strongly with Ste50 and more weakly with Ste11, which binds to Ste50 (B29). We believe that Ste50 is the target of the peptamer, because *ste50* (Delta) cells show only modest a factor resistance, even in the presence of the peptamer, whereas expressing the peptamer in wild-type cells conferred strong resistance (Fig. 4). Other peptamers caused strong pheromone resistance in *ste50* (Delta), showing that these cells can become fully pheromone resistant. This result suggests that Ste50 has positive and negative functions in signaling and that the peptamer stimulates the activity of Ste50 that interferes with signaling, possibly by stimulating adaptation of the signaling pathway (B30). One peptamer interacted with two MAP kinases (Fus3 and Kss1), either of which can transmit the pheromone signal, but failed to block the response to high osmolarity, which depends on the MAP kinase Hog1 (Table 2). This observation shows that peptamers are capable of discriminating between members of a family of homologous proteins.

Genetic selections can identify peptamers that inhibit biological pathways. Both of our selections produced two classes of peptamers, those that interfered indirectly with the target pathway and those that did so directly. Thus we obtained inhibitors of Mps1 expression as well as those that directly inhibit the spindle checkpoint and inhibitors of transcriptional silencing and those that interfere directly with pheromone signaling. These results highlight the importance of secondary tests that provide independent information about how each inhibitor passed the

original selection.

We used three different methods to gain information about the targets of the peptamers: two-hybrid analysis, transcript arrays, and pathway dissection. Each approach has strengths and weaknesses, and a combination of methods is likely to be the most reliable way to identify targets. Two-hybrid analysis is simple, reports on physical interactions, and can identify additional components of existing pathways. The major drawbacks are false positives and false negatives: 7 of the 15 peptamers we tested failed to give a reproducible interaction with any protein, 3 peptamers showed interactions with more than one protein, and, in two cases, genetic tests suggested that none of the interacting proteins was the physiological target of the peptamer. Analyzing transcript arrays tests whether peptamers produce a similar response to known genetic perturbations and is the most powerful way of analyzing the specificity of inhibition. However, if mutations in multiple genes produce a similar transcriptional response, the product of any one of these genes could be the target of a peptamer. Genetic tools that dissect a pathway into separate modules are good reagents for directly identifying the targets of peptamers, but they are available for only a small number of well-studied pathways. Other methods that may identify targets include screening for proteins whose overexpression overcomes the inhibitory phenotype and mass spectrometry-based identification of proteins that bind to peptamers.

Genetic selection of inhibitory peptamers has four possible applications to drug discovery. First, by selecting for proliferation of the cells in which the target pathway has been inhibited, it provides a useful minimum of specificity. Second, it identifies new targets for drug discovery by finding new components of the target pathway. Third, a protein whose function can be inhibited with a short peptide is likely to be a good candidate for inhibition by small organic molecules. Finally, genetic selections make it easy to isolate peptamers with higher and lower potencies, and correlations between structure and activity could be used to guide combinatorial or peptidomimetic chemistry.

#### Figure F1

Caption: Peptamer expression in yeast. Measurement of peptamer expression in yeast by Coomassie staining (left) and antihemagglutinin immunoblotting (right). Double-headed arrow denotes staphylococcal nuclease; double-headed closed circle denotes peptamer P-7. Numbers on left are kilodaltons.

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#### Figure F2

Caption: Identification of an inhibitor of the spindle checkpoint. (A) Effects of three peptamers on the spindle assembly checkpoint activated by overexpression of Mps1. Cultures of logarithmic-phase controls and inhibitor-containing strains (B15) were equalized for cell density, serially diluted by a factor of three, spotted onto -uracil/+galactose plates, and incubated at 30.Deg.C. GAL-MPS1 resistance was also measured by plating equal numbers of logarithmic-phase cells onto -uracil/+glucose and -uracil/+galactose plates and incubating at 30.Deg.C for 4 days. CDC20-127 is a dominant mutant that inactivates the spindle checkpoint (B33) and serves as a positive control. Percent resistance is defined as 100 times the number of colonies on the -uracil/+galactose plate divided by the number of colonies growing on the -uracil/+glucose plate. (B) Reduced amounts of Mps1 expression in peptamer-containing strains. To avoid cell-cycle-regulated changes in the stability of Mps1, we arrested all cells in mitosis with the cdc23-1 mutant, which lacks anaphase-promoting complex activity at high temperatures. Haploid strains (GAL-MPS1::URA3, cdc23-1) containing control or inhibitory peptamers were grown at 23.Deg.C in -uracil/+raffinose to early logarithmic phase and then shifted to 34.Deg.C in -uracil/+galactose for 4 hours to induce transcription of Mps1. Extracts were prepared and analyzed by protein immunoblotting with antibodies to Myc epitope-tagged Mps1. Numbers on left are kilodaltons. (C) Spindle checkpoint inhibition by peptamer C-3. Exponentially growing cells containing a control peptamer or inhibitor C-3 were arrested in G<sub>1</sub> by treatment with a factor for 4 hours. After a factor was washed out, cells were (i) sonicated briefly, incubated on -uracil/+benomyl (20 (μ) g/ml) plates, and scored for the percentage of cells that rebud after 5 hours at 16.Deg.C; or (ii) treated with nocodazole (15 (μ) g/ml) for 0, 2, 3.3, and 5 hours and plated for viability. (Delta) , Staphylococcal nuclease;

x , CDC20-127; (square-solid) , peptamer C-3. (D) Effect of deleting the YDR517w gene on the spindle checkpoint. Wild-type (WT) cells, wild-type cells plus a control peptamer or peptamer C-3 (which binds Ydr517w), and YDR517w (Delta) cells were tested for their ability to rebud in the presence of benomyl as in (C).

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### Figure F3

Caption: Identification of a factor-resistant peptamers that inhibit transcriptional silencing. (A) Cartoon of the pheromone response pathway in budding yeast. (B) Comparisons of the transcript array profiles of two silencing inhibitors and a dominant-negative mutant of the silencing protein Sir4. RNA samples were prepared from wild-type cells and from cells expressing peptamer S-1, S-5, or a dominant-negative allele of SIR4 and were analyzed by competitive hybridization to DNA microarrays containing >6200 yeast ORFs (B34) . Correlation plots comparing the results of these hybridizations are shown (B35) . (Top) Log.inf(10) of the expression ratio of ORFs from cells containing or lacking peptamer S-1 [(log.inf(10) (R/G)] plotted versus the log.inf(10) ratio of ORFs from cells containing or lacking peptamer S-5 expression. (Bottom) Log.inf(10) (R/G) ratios of ORFs from cells containing or lacking peptamer S-1 expression plotted versus ORFs from cells with or without SIR4.sup(DN) expression. For each plot, >5600 ORFs (91 to 96% of total spots) for which reliable data were measured are plotted. ORFs whose expression did not change in either experiment are plotted as gray dots; ORFs that changed significantly ( $P \leq 0.01$ ) in expression in both experiments are plotted as red stars; ORFs that changed significantly ( $P \leq 0.01$ ) only in the competitive hybridization plotted on the x or y axis are plotted as green or blue stars, respectively. Specific subtelomeric, MATa-, and haploid-specific genes are labeled. Supplementary material is available at: [www.sciencemag.org/feature/data/1041079.shl](http://www.sciencemag.org/feature/data/1041079.shl)

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Begin Table : Columns 1 - 5 of 5

### Caption:

Amino acid sequence Reference B31 and characterization of 20 silencing inhibitors. NA, not applicable; NI, no interaction detected; NT, not tested.

Gene on plasmid	Amino acid sequence	a-Factor Resistance (%)	Induces sporulation	Two-hybrid interaction
Staph nuclease	-	0	No	NA
MATa	-	100	Yes	NA
SIR4DN	-	100	Yes	NA
Inhibitor S-1 (7 aa)	VCLGGVP	82	Yes	NT
Inhibitor S-2 (15 aa)	RFFWNPWTRVMQRAP	66	Yes	NT
Inhibitor S-3 (4 aa)	WVNW	43	No	NT
Inhibitor S-4 (15 aa)	RRTGGWGGNTCIKFD	9	No	NT
Inhibitor S-5 (4 aa)	WVGW	70	No	DIG1, TEC1, STE12
Inhibitor S-6	VYLRKFSKVPITGW	59	No	NT
Inhibitor S-7 (9 aa)	VVWLDCW	100	Yes	DIG1, TEC1
Inhibitor S-8 (6 aa)	GRMEPGAAPRDSKCNA	49	No	NT
Inhibitor S-9	SLLATRS AKLALCSAR	91	Yes	NT
Inhibitor S-10	ILIKSKMHQRTLFSAL	100	Yes	SFH1
Inhibitor S-11	VYWRGQSLYATLSTSE	94	Yes	ASF1
Inhibitor S-12	VPSLRALWAYAGLGDS	79	Yes	NT
Inhibitor S-13	PCLVSSGPAGRSPSAW	11	Yes	NT
Inhibitor S-14	VYRCGPGGVLYPPACR	72	No	NT
Inhibitor S-15	PLLDPPQQAAPVAAGP	92	No	NI
Inhibitor S-16	ILLTRVHLRRSYMGAT	18	Yes	NT

Inhibitor S-17	FVFARRGYHLASTVHT	59	No	NT
Inhibitor S-18	CVACGLKLAGRLVGYL	85	No	NT
Inhibitor S-19	LLWSSVVKNPKFGGLF	100	No	NT
Inhibitor S-20	RLMSWRDSLWSYARLS	83	Yes	NT

Footnote:

Equal numbers of logarithmic-phase cells were plated on -uracil/+glucose plates either lacking or containing a factor (1 (mu) g/ml) and incubated at 30.Deg.C for 3 days. Percent resistance is 100 times the number of colonies on medium with a factor divided by the number of colonies growing on the plate lacking a factor.

Footnote:

A MATa/MATa strain was transformed with control or inhibitory peptamers, grown to saturation in -uracil/+glucose, and transferred to sporulation medium (2% KOAc, 0.02% raffinose) and incubated at 23.Deg.C for 3 days. Cells were analyzed under a microscope for the presence of tetrads.

Footnote:

Five peptamers were fused to a DNA binding domain and tested for two-hybrid interactions against a panel containing >85% of the ORFs in yeast fused to a transcriptional activation domain Reference B32 .

End Table: Columns 1 - 5 of 5

Begin Table : Columns 1 - 7 of 8

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Caption:

Amino acid sequence and characterization of nine pheromone signaling inhibitors.

Gene on plasmid	Amino acid sequence	a-factor Resistance (%)	At or From below Ste4	Ste11 to Fus1	At Ste11 , Ste20 , or Ste50	At or below Far1
Staph nuclease	-	0	NA	NA	NA	NA
MATa	-	100	NA	NA	NA	NA
SIR4DN	-	100	NA	NA	NA	NA
Inhibitor P-1	LYATRGLVRSHVCLGL	44	Yed	Yes	No	No
Inhibitor P-2	LLWSSVVKNPKFRHLF	100	Yes	No	No	No
Inhibitor P-3	WWVRREIWFGAVISYE	42	?	No	No	No
Inhibitor P-4	CRSVKEALVVFRMLQ	100	Yes	Yes	No	No
Inhibitor P-5	RIKGRYLAFVRQVGGF	51	Yes	No	No	No
Inhibitor P-6	CWVCVPRVLRQRLGI	76	Yes	No	No	No
Inhibitor P-7	VLDVKDASDESILLSW	100	Yes	Yes	Yes	No
Inhibitor P-8	HGGVPGRPPSFILWKM	75	Yes	Yes	No	No
Inhibitor P-9	EIRRWQATYPLFASS	76	Yes	Yes	No	No

Footnote:

See Table 1<TBLR RID="T1"> for determination of percent a factor resistance.

Footnote:

Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of Ste4 (pGAL-STE4).

Footnote:

Ability of peptamers to overcome the signaling of a constitutively active STE11-1 mutant. Cells containing a FUS1-HIS3 reporter induced by STE11-1 were transformed with control or inhibitory peptamers and tested for their ability to grow on -uracil/-histidine plates.

Footnote:

Osmotic sensitivity of a strain that depends on Ste11, Ste20 and Ste50 for osmotic signaling. A haploid MATa ssk1 strain Reference B28 that uses the Sho1 osmosensor and Ste11, Ste20, and Ste50 for osmoresistance was transformed with control or inhibitory peptamers. Transformants were tested for osmosensitivity by streaking onto -uracil/0.9 M NaCl plates.

Footnote:

Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of a dominant FAR1-22 mutant.

Footnote:

See legend to Table 1<TBLR RID="T1">.

## Caption:

Amino acid sequence and characterization of nine pheromone signaling inhibitors.

Gene on plasmid Two-hybrid  
interaction

Staph nuclease	NA
MATa	NA
SIR4DN	NA
Inhibitor P-1	FUS3, KSS1
Inhibitor P-2	NI
Inhibitor P-3	NT
Inhibitor P-4	NI
Inhibitor P-5	NT
Inhibitor P-6	NI
Inhibitor P-7	STE11, STE50
Inhibitor P-8	NI
Inhibitor P-9	ARG80

## Footnote:

See Table 1<TBLR RID="T1"> for determination of percent a factor resistance.

## Footnote:

Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of Ste4 (pGAL-STE4).

## Footnote:

Ability of peptamers to overcome the signaling of a constitutively active STE11-1 mutant. Cells containing a FUS1-HIS3 reporter induced by STE11-1 were transformed with control or inhibitory peptamers and tested for their ability to grow on -uracil/-histidine plates.

## Footnote:

Osmotic sensitivity of a strain that depends on Ste11, Ste20 and Ste50 for osmotic signaling. A haploid MATa ssk1 strain Reference B28 that uses the Sho1 osmosensor and Ste11, Ste20, and Ste50 for osmoresistance was transformed with control or inhibitory peptamers. Transformants were tested for osmosensitivity by streaking onto -uracil/0.9 M NaCl plates.

## Footnote:

Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of a dominant FAR1-22 mutant.

## Footnote:

See legend to Table 1<TBLR RID="T1">.

End Table: Columns 8 - 8 of 8

## Figure F4

Caption: Genetic identification of a peptamer target. Cultures of logarithmic-phase MATa, ste11 (Delta) MATa, or ste50 (Delta) MATa cells expressing staphylococcal nuclease, peptamer P-7, or peptamer S-7 were equalized for cell density, serially diluted by a factor of five, and spotted onto plates containing yeast extract, peptone, and dextrose (YPD) and a factor (1 (mu) g/ml) and incubated at 30.Deg.C for 3 days.

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16. For both selections, transformants were grown on uracil-deficient plates for 2 days. Cells were scraped from these plates, and aliquots were plated onto selection plates [lacking uracil and containing galactose (-uracil/+galactose) for the spindle checkpoint selection; lacking uracil and containing a factor (-uracil/+a factor) (1 ( $\mu$ ) g/ml) for the pheromone pathway selection] and grown at 30.Deg.C for 2 or 3 days. Plasmid DNA was recovered from the entire population of colonies that grew on these plates, purified, amplified by transformation into *E. coli*, and retransformed into DA2050A to enrich for peptamers that conferred resistance as opposed to genomic mutations that caused resistance. Cell populations harboring active peptamers typically grew as lawns on selection plates, and individual plasmids were isolated from these plates and retested to confirm that they gave plasmid-dependent resistance. ;
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36. Supported by grants from NIH, Human Frontier Science Program, and Chiron (A.W.M.); an NIH senior fellowship (T.C.N.); an NIH grant and the Herbert W. Boyer Fund (S.M.O.); and NIH grant P41-RR11823 (B.L.D. and S.F.). S.F. is an Investigator of the Howard Hughes Medical Institute. We thank L. Hartwell, I. Herskowitz, L. Huang, and J. Rine for yeast strains and plasmids; T. Geyer, Alejandro Colman-Lerner, and R. Brent for communicating unpublished results; and D. Gottschling, I. Herskowitz, L. Pillus, J. Simon, and members of the Seattle Project and the Murray laboratory for valuable discussions

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**Identification of genes encoding two-component lantibiotic production in *Staphylococcus aureus* C55 and other phage group II *S. aureus* strains and demonstration of an association with the exfoliative toxin B gene**

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The production of exfoliative toxin B (ET-B), but not ET-A, was shown to be specifically associated with production of a highly conserved two-component lantibiotic peptide system in **phage** group II **Staphylococcus aureus**. Two previously studied but incompletely characterized *S. aureus* bacteriocins, **staphylococcins** C55 and BacR1, were found to be members of this **antibiotic** system, and considerable homology was also found with the two-component *Lactococcus lactis* bacteriocin, lacticin 3147. *sac*alphaA and *sac*betaA, the structural genes of the lantibiotics **staphylococcins** C55alpha and C55beta and two putative lantibiotic processing genes, *sac*M1 and *sac*T, were localized together with the ET-B structural gene to a single 32-kb plasmid in strain C55. Irreversible loss of both ET-B and two-component lantibiotic production occurs during laboratory passage of ET-B-positive *S. aureus* strains, particularly at elevated temperatures.

SPECIES DESCRIPTORS:  
*Staphylococcus aureus*

CLASSIFICATION CODE AND DESCRIPTION:

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**96th General meeting of the American Society for Microbiology**

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LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The 96th Annual Meeting of the American Society for Microbiology covered an eclectic blend of presentations including: old therapeutic drugs looking for new recommendations, new drugs in the process of approval or in various phases of clinical trials, new developments in bacterial pathogenesis, protein secretion and immunomodulation.

BRAND NAME/MANUFACTURER NAME: augmentin/smith kline beecham/United States; unasyn/pfizer; ly 264826

MANUFACTURER NAMES: nexstar/United States; smith kline beecham/United States; pfizer

DRUG DESCRIPTORS:

\*antiinfective agent--drug therapy--dt; \*beta lactam **antibiotic** --drug therapy--dt; \*beta lactam **antibiotic** --clinical trial--ct; \*beta lactam **antibiotic** --drug dose--do; \*cytokine; \*glycopeptide--drug development--dv; \*macrolide; \*quinoline derived antiinfective agent--drug therapy--dt; \*quinoline derived antiinfective agent--drug dose--do; \*quinoline derived antiinfective agent--drug administration--ad  
amikacin; amoxicillin plus clavulanic acid--drug therapy--dt; amoxicillin plus clavulanic acid--drug dose--do; amoxicillin plus clavulanic acid --clinical trial--ct; azithromycin; beta lactamase; cefoxitin; chloroercentin a--drug development--dv; ciprofloxacin--pharmacokinetics --pk; ciprofloxacin--drug comparison--cm; ciprofloxacin--drug therapy--dt; erythromycin; gamma interferon--drug development--dv; granulocyte colony stimulating factor--drug development--dv; granulocyte colony stimulating factor--drug dose--do; immunomodulating agent; interleukin 12--drug development--dv; interleukin 13--drug development--dv; levofloxacin--drug administration--ad; levofloxacin--drug dose--do; levofloxacin--drug therapy

--dt; levofloxacin--pharmacokinetics--pk; levofloxacin--drug comparison--cm  
; liposome; metenkephalin; new drug; protamine; sultamicillin; teicoplanin;  
trovafloxacin--clinical trial--ct; trovafloxacin--drug dose--do;  
trovafloxacin--drug therapy--dt; unindexed drug; vancomycin--drug  
concentration--cr; zidovudine

MEDICAL DESCRIPTORS:

\*bacterial infection--drug therapy--dt; \*mycobacteriosis--drug therapy--dt;  
\*mycosis--drug therapy--dt

animal experiment; animal model; **antibiotic resistance**; **bacteriophage** ;  
bone infection--drug therapy--dt; clinical trial; conference paper;  
controlled study; dose response; double blind procedure; drug half life;  
drug screening; enterococcus; human; immunomodulation; intramuscular drug  
administration; intraperitoneal drug administration; minimum **inhibitory**  
concentration; mouse; multicenter study; nonhuman; phase 3 clinical trial  
; protein secretion; pseudomonas aeruginosa; randomized controlled trial;  
skin infection--drug therapy--dt; **staphylococcus** ; subcutaneous drug  
administration

CAS REGISTRY NO.: 37517-28-5, 39831-55-5 (amikacin); 74469-00-4 (  
amoxicillin plus clavulanic acid); 83905-01-5 (azithromycin); 9073-60-3  
(beta lactamase); 33564-30-6, 35607-66-0 (cefoxitin); 118395-73-6 (  
chloroercenticin a); 85721-33-1 (ciprofloxacin); 114-07-8, 70536-18-4 (  
erythromycin); 82115-62-6 (gamma interferon); 138415-13-1 (interleukin  
12); 148157-34-0 (interleukin 13); 100986-85-4, 138199-71-0 (  
levofloxacin); 58569-55-4 (metenkephalin); 11061-43-1, 9007-31-2,  
9012-00-4 (protamine); 76497-13-7 (sultamicillin); 61036-62-2,  
61036-64-4 (teicoplanin); 146836-84-2 (trovafloxacin); 1404-90-6,  
1404-93-9 (vancomycin); 30516-87-1 (zidovudine)

SECTION HEADINGS:

030 Clinical and Experimental Pharmacology  
037 Drug Literature Index

26/9/4 (Item 4 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

10031322 99028371 PMID: 9810676

**Characteristics of Staphylococcus aureus strains isolated from clinical  
and non-clinical human sources in Trinidad: susceptibility to  
bacteriophages and antimicrobial agents, and toxigenicity.**

Adesiyun A A; Prabhakar P; Ali C; Lewis M

Faculty of Medical Sciences, University of the West Indies, St.  
Augustine, Trinidad.

Zentralblatt fur Bakteriologie : international journal of medical  
microbiology (GERMANY) Oct 1995, 282 (4) p519-32, ISSN 0934-8840

Journal Code: 9203851

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The susceptibility of Staphylococcus aureus strains isolated from human  
clinical and non-clinical sources in Trinidad to bacteriophages and  
antimicrobial agents was determined. The ability of the strains to produce  
enterotoxins and toxic shock syndrome toxin-1 (TSST-1) was also  
investigated. Of the 554 strains tested, 454 (81.8%) were susceptible to  
international phage set (IPS) **phages** with strains isolated from  
bacteruria (57.1%) and bacteremia (53.3%) having a low sensitivity  
compared to isolates from aspirates (87.3%) and anterior nares (97.4%).  
All sources combined, strains were most susceptible to **phages** belonging  
to several groups (mixed). Overall, 419 (75.6%) strains were resistant to  
one or more of nine **antimicrobial** agents tested. Resistance to penicillin  
was most prevalent, with 413 (74.5%) strains found to be resistant.  
Prevalence of resistance to tetracycline, gentamicin, oxacillin, cefuroxime  
and ciprofloxacin was 5.1%, 2.0%, 0.7%, 0.4% and 0.4%, respectively. Of the  
554 strains tested, 307 (55.4%) produced **staphylococcal** enterotoxins A  
(SEA), B (SEB), C (SEC) and D (SED) singly or in combination. Strains  
recovered from high vaginal swabs were least enterotoxigenic (40.0%) as  
compared to umbilical infection isolates which were most enterotoxigenic  
(78.9%). TSST-1 was produced by 95 (19.0%) out of 499 strains tested, with

isolates from bacteruria found to be most toxigenic (33.3 %). It was concluded that the *S. aureus* strains tested were highly susceptible to **bacteriophages** and **antimicrobial** agents (except penicillin) and that enterotoxigenic and TSST-1 producers were widespread and have an aetiologic potential.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: \*Antibiotics--pharmacology--PD; \*Bacteriophages--physiology--PH; \*Enterotoxins--biosynthesis--BI; \*Staphylococcus aureus; \*Staphylococcus aureus--metabolism--ME; Staphylococcal Infections--microbiology--MI; Staphylococcus aureus--drug effects--DE; Staphylococcus aureus--virology--VI; Trinidad and Tobago

CAS Registry No.: 0 (Antibiotics); 0 (Enterotoxins); 0 (enterotoxin F, Staphylococcal); 12788-99-7 (enterotoxin D, Staphylococcal); 37337-57-8 (enterotoxin A, Staphylococcal); 39424-53-8 (enterotoxin B, staphylococcal); 39424-54-9 (enterotoxin C, staphylococcal)

Record Date Created: 19990122

26/9/14 (Item 14 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

10792597 20323749 PMID: 10865428

[Evaluation of the usefulness of new international experimental phages for typing methicillin resistant *Staphylococcus aureus* (MRSA)]

Ocena przydatnosci nowego, miedzynarodowego zestawu eksperymentalnych fagow do typowania opornych na metycyline gronkowcow zlocistych (MRSA).

Piechowicz L; Wisniewska K; Galinski J

Medycyna doswiadczalna i mikrobiologia (POLAND) 1999, 51 (1-2) p31-6, ISSN 0025-8601 Journal Code: 0210575

Document type: Journal Article ; English Abstract

Languages: POLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The aim of the study was to determine the usefulness of the set of experimental phages obtained from the Central Public Health Laboratory in London for typing of MRSA strains in Poland. The study was performed on 150 MRSA strains isolated from various clinical materials in various regions of the country. The set of 10 experimental phages and the international basic set of 23 phages were used for typing. The results of the study showed that 76.8% of MRSA strains were typing with the experimental set of **phages**. The frequency of **inhibition** reactions was 19.9%. Only 3.3% of the strains were nontypable with the new **phages** while nearly half of the studied strains were nontypable with the basic set of **phages**. The studied strains were divided into 19 phagotypes. There was a high frequency of typable strains among MRSA typable and nontypable strains and those **inhibited** by the basic set of **phages** (71.4%-85.7%). These data indicate that the set of 10 experimental **phages** is useful for typing of MRSA strains isolated in Poland except for **phage** M3 which failed to react with almost all the strains and should be excluded from the proposed set.

Descriptors: \*Bacteriophage Typing--methods--MT; \*Bacteriophages--classification--CL; \*Methicillin Resistance; \*Staphylococcus aureus--classification--CL; \*Staphylococcus aureus--drug effects--DE; Species Specificity

Record Date Created: 20000925

26/9/39 (Item 39 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

10053779 99028817 PMID: 9812283

**Protein antimicrobial barriers to bacterial adhesion.**

Bower C K; Daeschel M A; McGuire J

Department of Bioresource Engineering, Oregon State University, Corvallis, USA.

Journal of dairy science (UNITED STATES) Oct 1998, 81 (10) p2771-8, ISSN 0022-0302 Journal Code: 2985126R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The ability of microorganisms to adhere to solid surfaces is a problem of high visibility and has been the focus of numerous investigations because these organisms can cause disease and food spoilage. During the last several years, considerable attention has been focused on the development of food-grade **antimicrobial** barriers to adhesion in order to **inhibit** the initial adhesion of microbial contaminants by application of an **antimicrobial** agent to the surface rather than trying to remove these contaminants once they are adhered. The premise is that, if both the presence of the agent and its **antimicrobial** activity are maintained at the interface, sensitive bacterial cells or spores that attempt to attach would be killed. Nisin has been used in foods as a direct additive to **inhibit** the growth of Gram-positive cells and spores. Similarly, hen lysozyme is a commercially available **antimicrobial** protein that offers application in food processing systems, but the mode of action of this enzyme differs from that of nisin. We have shown that nisin can adsorb to surfaces, maintain activity, and kill cells that have adhered. In addition, we have addressed questions relating to the short- and long-term stability of adsorbed nisin, the degree to which immobilized nisin can resist exchange with dissolved solution components, and the surface concentrations that are necessary to **inhibit** biofilm formation. More recently, we have focused on basic questions relating to molecular influences on **antimicrobial** activity at interfaces using synthetic mutants of **bacteriophage** T4 lysozyme and hen lysozyme in addition to nisin.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: \*Anti-Infective Agents; \*Bacterial Adhesion; \*Biofilms; \*Food Handling--methods--MT; \*Proteins; Adsorption; Antibiotics, Peptide; Bacteriophage T4--enzymology--EN; Circular Dichroism; Enterococcus faecalis --physiology--PH; Listeria monocytogenes--physiology--PH; Muramidase --chemistry--CH; Nisin--chemistry--CH; Proteins--chemistry--CH; Staphylococcus--physiology--PH

CAS Registry No.: 0 (Anti-Infective Agents); 0 (Antibiotics, Peptide); 0 (Proteins); 1414-45-5 (Nisin)

Enzyme No.: EC 3 .2.1.17 (Muramidase)

Record Date Created: 19990119

04198178 Genuine Article#: RM672 Number of References: 41

**Title: HETEROGENEOUS ENDOLYSINS IN LISTERIA-MONOCYTOGENES BACTERIOPHAGES -  
A NEW CLASS OF ENZYMES AND EVIDENCE FOR CONSERVED HOLIN GENES WITHIN  
THE SIPHOVIRAL LYSIS CASSETTES**

Author(s): LOESSNER MJ; WENDLINGER G; SCHERER S

Corporate Source: TECH UNIV MUNICH, FORSCHUNGSZENTRUM MILCH & LEBENSMITTEL  
WEIHENSTEP, INST MIKROBIOL/D-85350 FREISING//GERMANY/

Journal: MOLECULAR MICROBIOLOGY, 1995, V16, N6 (JUN), P1231-1241

ISSN: 0950-382X

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Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences

Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; MICROBIOLOGY

**Abstract:** *Listeria monocytogenes* bacteriophages A118, A500 and A511 are members of three distinct phage groups with characteristic host ranges. Their endolysin (ply) genes were cloned and expressed in *Escherichia coli* as demonstrated by the conferred lytic phenotype when colonies of recombinant cells were overlaid with a lawn of *Listeria* cells. The nucleotide sequences of the cloned DNA fragments were determined and the individual enzymes (PLY118, 30.8 kDa; PLY500, 33.4 kDa; PLY511, 36.5 kDa) were shown to have varying degrees of homology within their N-terminal or C-terminal domains. Transcriptional analysis revealed them to be 'late' genes with transcription beginning 15-20 min post-infection. The enzymes were overexpressed and partially purified and their individual specificities examined. When applied exogenously, the lysins induced rapid lysis of *Listeria* strains from all species but generally did not affect other bacteria. Using hydrolysis of purified listerial cell walls, PLY511 was characterized as an N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) and shows homology in its N-terminal domain to other enzymes of this type. In contrast, PLY118 and PLY500 were shown to represent a new class of cell wall lytic enzymes which cleave between the L-alanine and D-glutamate residues of listerial peptidoglycan; these were designated as L-alanoyl-D-glutamate peptidases. These two enzymes share homology in the N-terminal domain which we propose determines hydrolytic specificity. Highly conserved **holin** (hol) gene sequences are present upstream of ply118 and ply500. They encode proteins of structural similarity to the product of phage lambda gene S, and are predicted to be membrane proteins which form pores to allow access of the lysins to their peptidoglycan substrates. This arrangement of conserved **holin** genes with downstream lysin genes among the siphoviral lysis cassettes explains why the cytoplasmic endolysins alone are not lethal, since they require a specific transport function across the cell membrane.

**Identifiers--Keywords Plus:** LAMBDA-S-PROTEIN; RNA- **POLYMERASE** ; PHAGE PHI-29; SEQUENCE-ANALYSIS; *ESCHERICHIA-COLI*; ENCODING GENE; CLONED GENES; EXPRESSION; CLONING; BACTERIA

**Research Fronts:** 93-8060 002 (MEMBRANE DOMAIN OF A BACTERIOPHAGE ASSEMBLY PROTEIN; *ESCHERICHIA-COLI* K-12; SYNTHETIC GENE; AFRICAN SWINE FEVER VIRUS; PHI-29 DNA- **POLYMERASE** ACTIVE-SITE)

93-2115 001 (FERMENTATION OF THE PRODUCING STRAIN; PRADIMICIN DERIVATIVES; ANTIFUNGAL ACTIVITY; DEVELOPMENTAL THERAPEUTICS; CORYNEFORM BACTERIA)

93-3981 001 (*LISTERIA-MONOCYTOGENES* SURVIVAL IN WHITE PICKLED CHEESE; COLORIMETRIC NUCLEIC-ACID HYBRIDIZATION ASSAY; ENRICHED CULTURES OF INOCULATED FOODS)

93-4847 001 (HETEROLOGOUS EXPRESSION; CHROMOSOMAL DNA; GENE ENCODING METHYLMALONYL-COENZYME-A MUTASE)

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32/9/5 (Item 5 from file: 155)  
 DIALOG(R) File 155:MEDLINE(R)

09814013 98232107 PMID: 9572396

**The lambda holin accumulates beyond the lethal triggering concentration under hyperexpression conditions.**

Smith D L; Chang C Y; Young R Y

Department of Biochemistry and Biophysics, Texas A&M University, College Station 77843, USA.

Gene expression (UNITED STATES) 1998, 7 (1) p39-52, ISSN 1052-2166  
 Journal Code: 9200651

Contract/Grant No.: GM27099; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Most bacteriophages terminate infection by creating lesions in the cytoplasmic membrane, which not only cause immediate cell death but also allow escape of a phage-encoded endolysin. Destruction of the peptidoglycan and cell lysis follows very rapidly, allowing efficient release of the progeny virions. These membrane lesions are formed by a small integral membrane protein called a **holin**. **Holins** have highly charged carboxyl-termini that are thought to have two transmembrane alpha-helical domains. **Holins** are believed to oligomerize and form large holes in the inner membrane. The prototype **holin** is the S protein from bacteriophage lambda. Scheduling of the lytic event is determined in part by the "structure directed initiation" or sdi translational control region. Inductions of S, cloned under a variety of native and nonnative promoters but with native translational control, resulted in cell lysis at about 1000 molecules of **holin** per cell, and thus do not produce biochemically useful amounts of S protein. By utilizing a plasmid-based system with the T7 RNA polymerase promoter in tandem with a consensus ribosome binding site, Coomassie blue-detectable quantities of S protein were obtained upon

**Antisense RNA-regulated programmed cell death.**

Gerdes, Kenn

Gultyaev, Alexander P; Franch, Thomas

Annual Review of Genetics (Annu Rev Genet) v. 31 ('97) p. 1-31

SPECIAL FEATURES: bibl il ISSN: 0066-4197

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

RECORD TYPE: Abstract; Fulltext RECORD STATUS: Corrected or revised record

WORD COUNT: 12418

**ABSTRACT:** The characteristics of the hok gene family were examined. These plasmid-encoded killer genes are responsible for plasmid stabilization by killing plasmid-free cells. The details of the complicated antisense RNA-regulated control loop that controls posttranscriptional and postsegregational activation of killer mRNA translation in plasmid-free cells are discussed. Nucleotide covariations in the mRNAs identify metastable stem-loop structures that form at the mRNA 5' end in the nascent transcripts and prevent translation and antisense RNA binding during transcription. Coupled nucleotide covariations are evidence of a phylogenetically conserved mRNA folding pathway that involves sequential dynamic RNA rearrangements. Thus, there is an intricate mechanism that allows for the conditional activation of translation of an antisense RNA-controlled mRNA. The complex phylogenetic relationships of the plasmid- and chromosome-encoded systems are also described.

**TEXT:**

**KEY WORDS**

hok, sok, RNA rearrangements, RNA folding pathway, cell killing

**INTRODUCTION**

Bacterial plasmids are inherited independently of the host cell chromosome. Despite their extrachromosomal status, bacterial plasmids are stably maintained. The stable maintenance may be due to a high copy number of the elements. However, in low-copy-number plasmids, stable inheritance depends on the presence of a number of gene systems that, by different mechanisms, actively prevent the appearance of plasmid-free progeny (28, 38, 61). Unitcopy plasmids such as F, P1, and R1 encode centromere-like functions that are thought to equi-partition the plasmid molecules at cell division (11, 61). The group of proteic plasmid stabilization systems constitutes a second type of maintenance systems (42). These latter systems mediate plasmid stabilization by killing plasmid-free segregants. The proteic systems specify two protein components: a stable toxin and an unstable antidote. The antidotes neutralize their cognate toxins by forming tight complexes with them. Since the antidotes are labile, newborn plasmid-free cells experience decay of the antidotes. This activates the toxins that, in turn, kill the plasmid-free cells (42). The proteic systems were originally identified on plasmids (6, 47, 53, 64, 80, 88), and a number of chromosomal counterparts were subsequently discovered (29, 51, 89). The function of the chromosome-encoded systems is unknown, but they appear to be dispensable for cell growth and viability under laboratory conditions (51). Recent findings indicate that plasmid-encoded restriction-modification systems mediate plasmid stabilization (46, 56). The stabilization phenotype was found to be due to killing of plasmid-free cells. The selective killing is a consequence of the dilution of the modification enzyme in growing plasmid-free cells. Below a certain level, the modification enzyme cannot protect the host cell from detrimental cleavage by the restriction enzyme.

Some ten years ago, we discovered yet another type of plasmid-encoded system that mediates efficient plasmid stabilization by killing plasmid-free cells (24, 26). In these systems, the antidotes are antisense RNAs that inhibit translation of toxin-encoding mRNAs. The antisense RNAs are unstable, whereas the toxin-encoding mRNAs are very stable. The selective killing of plasmid-free cells is based on the differential decay rates of the antisense RNAs and the mRNAs. The prototype of these latter systems is hok/sok from plasmid R1. Thirteen hok-homologous genes from



eubacterial plasmids and chromosomes have been identified to date. Interestingly, the chromosome of *Escherichia coli* K-12 contains five such genes. In this review, we describe the genetics, molecular biology, and evolution of the *hok* gene family. We emphasize the complicated RNA biology that governs the postsegregational activation of *hok* mRNA translation in plasmid-free cells. As will become evident, our analyses have revealed a number of interesting surprises.

#### THE PROTOTYPE SYSTEM: HOK/SOK FROM PLASMID R1

The *hok/sok* system from plasmid R1 was discovered because it can stabilize the inheritance of plasmids replicating in *E. coli* (24). The stabilization is very efficient and replicon independent (4, 20, 24). Low-copy-number plasmids (F and R1) are stabilized 50- to 100-fold, whereas high-copy-number plasmids (p15A and pBR322) are stabilized 1000- to 10,000-fold. Thus, *hok/sok* stabilizes high copy-number-plasmids with such high fidelity that they are considered technically stable (97-99). The *hok/sok* system mediates efficient plasmid stabilization in a wide variety of Gram-negative bacteria (20, 22). Therefore, the *hok/sok* system constitutes a simple and efficient tool for the technical stabilization of bacterial plasmids in a wide variety of cases, as recently reviewed in Reference 22.

The genetic organization of the *hok/sok* system is shown in Figure 1A. The locus codes for three genes are denoted *hok*, *sok*, and *mok*, respectively. The *hok* (host killing) gene specifies a membrane-associated toxin of 52 amino acids (aa) (26). The toxin causes irreversible damage to the cell membrane and is thus lethal to host cells (21). The *sok* gene (suppression of killing) codes for an antisense RNA of 64 nucleotides (nt), which is complementary to the *hok* mRNA leader region (23, 27). *Sok*-RNA is unstable, but is constitutively expressed from a relatively strong promoter. In contrast, *hok* mRNA is very stable and is constitutively expressed from a relatively weak promoter (23, 27). The *mok* (modulation of killing) reading frame overlaps extensively with *hok*, and it is required for expression and regulation of *hok* translation (49, 83). Genetic analyses showed that *Sok*-RNA inhibits translation of the *mok* reading frame and that translation of *hok* is coupled to translation of *mok* (83). Consequently, *Sok*-RNA regulates translation of *hok* indirectly via *mok*. The secondary structure of the 64-nt *Sok*-RNA is shown in Figure 1B.

#### PLASMID STABILIZATION BY POSTSEGREGATIONAL CELL KILLING

In 1986, we investigated the mechanism of plasmid stabilization using a pSC101 test plasmid with a temperature-sensitive replication system (26). The advantage of such a system was that synchronous plasmid-curing could be obtained simply by shifting growing cells from 30[degree]C (at which temperature the pSC101 plasmid replicated normally) to 42[degree]C (which prevented further rounds of plasmid replication, but allowed normal cell growth and division). Using this technique, it was shown that cells cured of a plasmid carrying *hok/sok* are rapidly killed (26). The selective cell killing prevents the proliferation of plasmid-free progeny. Phenotypically, this leads to plasmid stabilization in a growing bacterial culture. This phenomenon was coined postsegregational killing, or PSK (26). The discovery of the PSK principle behind the plasmid-stabilization phenotype was exciting: The killing appeared paradoxical, since *Hok* activity was triggered in cells devoid of the *hok* gene, and furthermore, the killing could be regarded as a terminal differentiation event initiated by plasmid loss. Other examples of programmed cell death in bacteria have been described and reviewed recently (42, 100).

#### PLASMID-ENCODED HOK HOMOLOGOUS GENES

Conjugative low-copy-number plasmids belonging to the IncF, IncB, and IncI incompatibility groups (plasmids that cannot coexist stably in the same cell line are said to be incompatible) carry regions that cross-hybridize with *hok* probes in Southern analyses (32). Over the years, six of these systems have been cloned and analyzed. The main properties of these systems are listed in Table 1 and described in some detail below.

#### THE FLM LOCUS OF PLASMID F

The conjugation systems of R1 (IncFII) and F (IncFI) are similar, and heteroduplex analyses have shown that the sequence homology extends into the transfer leading regions of the plasmids (19, 96). The hok/sok system is located in the transfer leading region of plasmid R1, a few kilobases (kb) downstream of the origin of transfer (oriT). Thus, during conjugation, hok/sok is transferred early. A locus from the leading region of F (designated flm for F leading maintenance) was found to stabilize the inheritance of heterologous replicons (33, 50). By comparing DNA sequences, it appeared that the flm and hok/sok loci are 95[percent] similar and that the Hok and FlmA proteins only differ by one aa (28, 50). Curiously, a variant of the flm locus (called stm for stable maintenance) has an in-frame insertion in the toxin-encoding reading frame, resulting in a killer protein with seven additional aa near the C-terminal end (33). The origin of this "in-frame" insertion in the flmA gene is not known. As in hok/sok of R1, flm and stm of F stabilize heterologous plasmids (33, 50).

#### THE SRNB LOCUS OF PLASMID F

In 1972, it was observed that the addition of rifampicin to certain *E. coli* strains resulted in membrane damage, RNase I influx from the periplasm, and degradation of stable RNA (rRNA and tRNA) (69). This syndrome is similar to the cellular changes induced by the Hok protein (21) (see discussion below). The locus responsible for the rifampicin-induced degradation of stable RNA was designated srnB (stable RNA degradation) and was found to be encoded by the sex factor F (67). Comparison of hok/sok and srnB revealed that the two loci have a similar structural organization, similar regulatory elements, and identical phenotypes (28, 59). Thus, although the systems are [similar]40[percent] dissimilar at the nucleotide level, they appear to be functionally equivalent. This is surprising, given that F also carries the flm system.

#### THE PND LOCI OF R483, R16, AND R64

The pnd (promotion of nucleic acid degradation) loci of R483 (IncIa) and R16 (IncB) were, like srnB of F, identified by rifampicin-induced membrane damage (66). Later structural and functional analyses showed that the pnd loci belong to the hok gene family, mediate plasmid stabilization, and encode RNAs similar to those of hok/sok (28, 59). A pnd locus very similar to pnd of R483 was recently identified on the *Salmonella typhimurium* virulence plasmid R64 (62).

#### OTHER PLASMID-ENCODED HOK-HOMOLOGOUS LOCI

A number of other plasmid-encoded hok-homologous loci have been identified. Using Southern hybridization, it was shown that [similar]50[percent] of all IncF-like plasmids are homologous in their transfer leading regions and may therefore contain hok-like genes (32). The IncI plasmid ColIb-P9 contains a locus closely related to pnd of R483 (9). *E. coli* cells containing the plasmids R621a (IncIg) and R483 (IncB) exhibit the typical Hok-induced damage response after the addition of rifampicin (66). Therefore, these plasmids most likely also encode hok-like loci (i.e. pnd).

Taken together, these observations show that hok-homologous gene systems are surprisingly abundant on plasmids from enteric bacteria. To date, all the plasmid-encoded systems tested mediate PSK and membrane damage upon addition of rifampicin to growing cells (see Table 1).

#### POSTSEGREGATIONAL CELL KILLING RELIES ON DIFFERENTIAL RNA DECAY

It was of obvious interest to elucidate the control loop that regulates postsegregational killing by hok/sok and its homologues. The sok (suppression of killing) gene encodes a trans-acting antisense RNA that inhibits hok mRNA translation (23, 26). We measured the lifetimes of the hok- and sok-encoded RNAs and found that hok mRNA is exceptionally stable (half-life in the order of hours) and that Sok-RNA is quite labile (half-life in the order of 30 seconds). These basic observations led to the proposal that induction of Hok activity in plasmid-free cells or in cells treated with rifampicin relies on the differential decay rates of the hok- and sok-encoded RNAs: in both situations, the rapid decay of the

antisense RNA results in an uninhibited pool of toxin-encoding *hok* mRNA freely accessible for translation. Similar differential decay rates for the antisense RNAs and mRNAs have been observed in the cases of *flm*, *srnB*, and *pnd* systems (59). Thus, the proposed scheme in a simple way explains the killing of plasmid-free segregants and the induction by rifampicin (23, 27). Basically, this induction model is still valid. However, the events preceding activation of *hok* mRNA translation in plasmid-free cells are considerably more complicated and involve a unique type of RNA metabolism, as described in the following sections.

#### FULL-LENGTH HOK mRNA IS TRANSLATIONALLY INACTIVE

The PSK model proposed above is based on an antidote-toxin principle by which the selective killing of plasmid-free cells is explained by the instability of the antidote. However, the interaction between *Sok*-RNA and *hok*-mRNA leads to duplex formation between the RNAs. The duplexes are rapidly cleaved by RNase III both in vitro and in vivo (25). Thus, as described for numerous antisense RNA gene systems, the proposed scheme of inhibition leads to irreversible mRNA inactivation and decay (3, 7, 45, 92). Therefore, the initial PSK model did not explain how *hok* translation is activated in plasmid-free cells. The answer to this important paradox came from the observation that *hok* mRNA exists in two forms (25, 27). One form, denoted full-length *hok* mRNA, is translationally inactive and binds the antisense RNA very inefficiently (18, 25, 27, 84). The secondary structure of the inert full-length *hok* mRNA is shown in Figure 2B and was obtained by structure probings, mutational analyses, phylogenetic comparisons, and computer predictions (17, 18, 35, 58, 85). The 3' end specifies a so-called "fold-back-inhibition" element (*fbi*) that pairs with the very 5' end of the mRNA. The 5' to 3' pairing locks the RNA in an inert configuration in which the Shine-Dalgarno element of *hok* (*SDhok*) is sequestered by an upstream anti-SD element denoted *ucb* (upstream complementary box). The Shine-Dalgarno element of *hok* (*SDhok*) is sequestered by a nearly perfect repetition of the *ucb* element denoted *dcb* (downstream complementary box). The fold-back inhibitory element also sequesters the antisense RNA recognition element in *hok* mRNA (*sokT*), thus preventing antisense RNA binding (Figure 2B). Therefore, full-length *hok* mRNA is inert with respect to translation and antisense RNA binding. Since the mRNA is inert, it can accumulate without killing the host cells, and it simultaneously avoids inactivation due to antisense RNA binding. As described below, the accumulation of a reservoir of an activable full-length *hok* mRNA is a prerequisite for the postsegregational killing mechanism.

#### ACTIVATION OF TRANSLATION

##### TRANSLATION OF HOK IS ACTIVATED BY MRNA 3' PROCESSING

A second version of the *hok* mRNA is generated by slow 3' processing of the full-length mRNA (27). The 3' processing leads to removal of the 40 terminal nucleotides of the full-length mRNA (see Figure 2B, C). The truncated mRNA is stable, translationally active, and binds *Sok*-RNA avidly (18, 25, 27, 84).

*E. coli* cells contain two 3' exoribonucleases involved in mRNA metabolism, polynucleotide phosphorylase (PNPase), and ribonuclease II (34). Recently, we investigated the 3' processing of *hok* and *pnd* mRNAs using *E. coli* host cells mutated in either *pnp* (encoding PNPase) or *rnb* (encoding ribonuclease II), or both (ND Mikkelsen & K Gerdes, unpublished). Inactivation of either one of the exonucleases did not prevent the 3' processing, whereas the simultaneous inactivation of both enzymes abolished processing. Thus, PNPase and ribonuclease II can both accomplish the 3' processing of *hok* and *pnd* mRNAs. Usually, 3' processing by PNPase and ribonuclease II leads to mRNA inactivation and decay (10, 14, 52). Therefore, the *hok* family of mRNAs constitutes an unusual case in which the opposite is true: Translation is activated by 3' exonucleolytic processing.

##### THE 3' PROCESSING TRIGGERS STRUCTURAL REARRANGEMENTS AT THE MRNA 5'

END

Single point-mutations in the very 5' end of *hok* mRNA prevent translation of *hok* and therefore also translation of *hok* (17). This mutational

analysis defined a translational activation element (tac) at the 5' end of the mRNA (Figure 2B, C). The tac element is located [similar] 100 nt upstream of the SDmok element, which constitutes its target of regulation. Besides being complementary to the 3' fbi element, tac is also partly complementary to the ucb element that sequesters SDmok in full-length hok mRNA (Figure 2B). The 3' processing, which disrupts the fbi-tac pairing, might therefore trigger refolding of the mRNA 5' end such that tac pairs with ucb in the truncated mRNA. The refolding, in turn, could mediate rearrangements further downstream in the RNA with the formation of structures that would allow translation and antisense RNA binding. This proposal was supported by folding simulations by the so-called genetic algorithm (36). Using the structure of the full-length mRNA as the input (Figure 2B), the algorithm was asked to simulate the 3' processing by progressive shortening of the length of the RNA chain by a few nucleotides at each successive calculation of intermediate structures (for details, see Reference 35). Thus, the algorithm disrupted the fbi-tac interaction by stepwise shortening of the RNA chain until the 3' end of the truncated mRNA was reached. This type of analysis was accomplished for hok mRNA as well as for many of the other plasmid- and chromosome-encoded mRNAs.

It appeared that the simulated 3' processing predicted similar structural rearrangements of the 5' untranslated regions in all cases. The refolded, truncated hok mRNA is shown in Figure 2C. The partial tac-stem already present at the 5' end of the full-length mRNA was extended, thus giving rise to the entire tac-stem in which the very 5' end pairs with ucb. Thus, the refolding disrupted the ucb/SDmok pairing known to prevent translation (17, 58). The in vivo genetic analyses and the in vitro secondary structure analyses confirmed the presence of the tac-stems in the refolded hok and pnd mRNAs (18), and sequence comparisons support the hypothesis that all the hok-related mRNAs form similar tac-stems (see Figure 3).

#### STRUCTURE AND FUNCTION OF THE ANTISENSE RNA TARGET

The refolding of the 5' end of hok mRNA was accompanied by significant structural rearrangements in the translation initiation regions located downstream of the tac-stem. A stem-loop structure, denoted the antisense RNA target hairpin, appeared in the folding simulations (Figure 2C). Structural analyses confirmed the existence of the target hairpin (18), and sequence comparisons show that similar hairpins are present in all hok-related mRNAs (Figure 3).

In the target hairpin, SDmok is located at the bottom of the 5' part of the antisense RNA target-stem and is sequestered by the dcb element (Figure 2C). Usually, such sequestration leads to inhibition of translation (12, 13, 30). However, mutations in dcb, which disrupted the pairing, abolished translation of truncated hok mRNA (18). Structural analyses indicated that the dcb mutations changed the overall secondary structure of the truncated RNA in favor of the ucb/SDmok pairing. This indicates that the SDmok/dcb pairing in the target-stem is required to maintain truncated hok mRNA in a translatable configuration (18). This conclusion was corroborated by analyses in which the tac-stem was forced by mutation. In such engineered mRNAs, the dcb mutations activated translation dramatically (18).

All antisense RNA target-stems in the hok-homologous mRNAs are between 13 and 16 base-pairs (bp) long and topped by loops between 7 and 9 nucleotides (see Figure 3). More important, the loops are in all cases complementary to the very 5' ends of the cognate antisense RNAs. The nucleotides complementary to the very first nucleotide in the antisense RNAs are in all cases rCs located at similar positions in the loops (Figure 2C, Figure 3). The similarity of the stem-loops suggests that the cognate antisense RNAs recognize these structures in all cases.

#### PHYLOGENETICALLY CONSERVED FOLDING PATHWAY

##### METASTABLE HAIRPINS IN THE NASCENT TRANSCRIPTS

The tac-stem, which is required for activation of translation, is located at the 5' end of hok mRNA (Figure 2C). If formed in the nascent transcript, the tac-stem predictably would lead to formation of the antisense RNA target stem-loop further downstream, thereby leading to premature antisense RNA binding or to translation of hok. Obviously, both

these scenarios would be detrimental to the PSK mechanism. How, then, is premature tac-stem formation prevented?

It appears that *hok* mRNA can form a local 5' hairpin as an alternative to the tac-stem (Figure 2A) (35). Similar hairpins were found in all *hok*-homologous mRNAs, and their existence is strongly supported by nucleotide covariations that preserve base-pairing (Figure 3). Estimates of folding kinetics suggest that the 5' hairpins are long-lived and probably exist until completion of the transcripts (i.e. the hairpins are metastable). This suggests that the metastable hairpins persist until they are disrupted by the establishment of the *fbi*-tac interactions in the full-length transcripts (35).

Using phylogenetic comparisons and RNA folding simulations, we delineated a "consensus" folding pathway for the *hok* family of mRNAs (35). The main conserved intermediate structures of the folding pathway are visualized in Figure 2, using *hok* mRNA as an example. In the 5' untranslated region, the folding of the metastable hairpin at the very 5' end prevents tac-stem formation and favors the formation of the *ucb*/*SD**mok* and *dcb*/*SD**hok* interactions (Figure 2A). These hairpins are also present in the full-length mRNA (Figure 2B). Therefore, during mRNA synthesis, the antisense RNA target hairpin is not formed (Figure 2A versus 2C). Presumably, this simultaneously reduces the rate of antisense RNA binding and prevents ribosome loading to the nascent transcript (35).

#### FOLDING OF THE CODING REGIONS

The folding simulations predicted that the coding regions of all *hok*-homologous mRNAs fold into similar and very stable structures (35). Secondary structure analyses supported the presence of the predicted structure in *hok* mRNA (85). In all RNAs, the coding regions were closed by stable pairings between their 5' and 3' ends, thus representing separate domains of about 140 nucleotides in the secondary structures. The 5' to 3' pairing of the coding regions may facilitate the formation of the *fbi*-tac interactions in the full-length mRNAs (Figure 2B).

#### FOLDING OF THE FULL-LENGTH MRNAs

In full-length RNAs, the 5' tac-elements were predicted to pair with the 3' *fbi*-elements for all mRNAs. This interaction resulted in disruption of the metastable hairpins, and caused further refolding of the 5' regions with partial formation of the tac-stems. The refolded full-length *hok* mRNA is shown in Figure 2B. The *fbi*-tac pairings are strongly supported by nucleotide covariations in the RNAs (Figure 3) and were recently verified experimentally in the *hok* and *pnd* mRNAs (17, 18).

#### FOLDING OF THE TRUNCATED MRNAs

Folding simulations suggest that the 3' processing of full-length *hok* mRNA triggers a rearrangement of its 5' end with formation of the tac-stem and the antisense RNA target stem-loop (see Figure 2C) (35). The target stem-loop allows rapid antisense RNA binding or translation (in the absence of antisense RNA). Similar refolding events triggered by 3' processing were predicted for all plasmid-encoded *hok*-homologous mRNAs and for all chromosome-encoded mRNAs, except the *E. coli hokD* (*relF*) mRNA, whose 5' end is not homologous to those of the other mRNAs.

Direct experimental evidence was obtained for all the postulated structures in *hok* mRNA except in the case of the metastable structure (17, 18, 58, 85). However, the phylogenetic data presented below provide reliable support for the importance of the proposed transient foldings.

#### A LARGE NUMBER OF NUCLEOTIDE COVARIATIONS SUPPORT THE SECONDARY STRUCTURES

The alignment of 9 of the 13 known *hok*-homologous mRNAs is shown in Figure 3. Because of close similarities with other mRNAs of the family, *hokA* of *E. coli* K-12 and *pnd* of the *Salmonella typhimurium* plasmid R64 were omitted from the alignment. The *hokD* (*relF*) and *hokE* loci were omitted because they lack essential regulatory elements (see later). All known *hok*-homologous sequences are derived from *E. coli* plasmids or chromosomes, or from bacteria closely related with *E. coli*. Therefore their large

sequence diversity appears quite surprising. Furthermore, despite such diversity, the overall genetic organization of the systems has been conserved. This suggests that a strong evolutionary selection pressure ensures the overall structural conservation of the systems.

The alignment in Figure 3 clearly demonstrates the presence of the conserved, mutually exclusive secondary structures in the mRNAs. By close scrutiny of the aligned sequences, a large number of nucleotide covariations that support the postulated secondary structures were disclosed (35). Covarying bases (different bases that form base-pairs at the same position in similar stems) are shown in color in Figure 3. As seen from the Figure, all structures contain at least three covarying base-pairs. Two or more covariations in similar secondary structures of phylogenetically related RNAs are generally accepted as reliable proof for their existence (41, 60, 95). The nucleotide covariations in the metastable hairpins are particularly important, since their transient nature renders it relatively difficult to obtain direct chemical evidence for their existence.

**COUPLED COVARIATIONS YIELD STRONG SUPPORT FOR THE FOLDING PATHWAY**  
The sequence alignment further reveals that some nucleotides covary with two partners (called coupled covariations and shown in red in Figure 3). For example, the unique C residue in pnd at the position corresponding to U141 in hok (in the dcb element) is paired to unique Gs upstream (nt 106) in the antisense target stem-loop or downstream (nt 169) in the stem that blocks the SD-element of the toxin gene. Other covariations are even more complicated. For example, the covariation of pair 5-71 in the tac-stem is coupled with covariations of pairs 71-109 in the ucb/SDmok pairing and with pair 5-392 in the fbi-tac interaction. Taken together, the coupled covariations provide evidence that the 5' tac-elements pair with three different partners during the folding path as visualized in Figure 2: (a) in the metastable structures during transcription, (b) in the long-range fbi-tac interaction in full-length RNAs, and (c) with the ucb elements in the tac-stems in truncated RNAs. Furthermore, the coupled covariations show that these mutually exclusive structures coevolve such that the overall folding pathway is preserved (15).

#### **ANTISENSE RNA BIOLOGY** **SECONDARY STRUCTURE**

The secondary structure of Sok antisense RNA from plasmid R1 is shown in Figure 1B. The RNA consists of a 5' single-stranded leader of 11 nt followed by a hairpin with a stem of about 20 base-pairs with two looped-out nucleotides (86). The stem-loop is followed by several rU's, which may ensure termination of transcription. In addition, the stable stem proposedly prevents the single-stranded 5' end from interacting with other parts of the molecule, thereby ensuring that the 5' end is ready to interact with the target-loop in truncated hok mRNA. Computer analyses predict similar foldings for all the Sok-homologous antisense RNAs.

Figure 4 shows the alignment of the Sok-homologous antisense RNAs. The alignment reveals a number of covariations (shown in boldface) that support the proposed foldings of the RNAs. Thus, it can be concluded that all plasmid- and chromosome-encoded Sok-homologous antisense RNAs have similar secondary structures (Figure 1B), in accordance with previous work (59). In the antisense RNAs, the stems are the equivalents of (i.e. complementary to) the ucb/SDmok stems in the corresponding mRNAs.

**METABOLISM BY RNASES AND POLY(A) POLYMERASE I**  
The Sok-homologous antisense RNAs are characterized by high turnover rates with typical half-lives of less than one minute (27, 59). The instability is due to processing by intracellular RNases. Using *E. coli* host cells mutated in the rne gene (encoding RNase E), it was shown that Sok-RNA is functionally inactivated by RNase E-mediated endo-cleavage at its 5' single-stranded leader (54). The position of cleavage, 6 nt from the 5' end of Sok-RNA, is indicated in Figure 1B. RNase E cleaves RNA I from ColE1 5 nts from its 5' end, which is also in a single-stranded configuration (48, 87). Furthermore, as in the case of RNA I, Sok-RNA is polyadenylated by poly(A) polymerase I (PcnB) in vivo (54). The

polyadenylation renders the downstream RNase E cleavage product an efficient substrate for polynucleotide phosphorylase which rapidly degrades the RNA from its 3' end. Thus, the metabolism of Sok-RNA exhibits a striking resemblance to that of RNA I of ColE1 in all major aspects investigated (10).

#### MECHANISM OF ANTISENSE RNA BINDING

Antisense RNAs responsible for plasmid-replication control usually recognize their target RNAs via an initial loop-loop interaction (15, 73, 92). The initial complex (the so-called kissing complex) is rapidly converted to a more stable configuration that involves duplex formation between the two RNAs. Usually, a single-stranded region near the 5' end of the antisense RNA recognizes the complementary region in the target RNA, and from there on more complete duplexes are formed. Thus this type of antisense RNA-binding mechanism involves two steps. In contrast, Sok-RNA recognizes its target RNA via its 5' single-stranded region by a one-step binding mechanism (18, 86). The structure of the antisense RNA target stem-loop in hok mRNA is shown in Figure 2C. The nucleotides in hok mRNA that are complementary to the very 5' end of Sok-RNA are presented in a loop of 7 nt (the target-loop) and are thus probably in a configuration that can be recognized by the single-stranded 5' end of Sok-RNA (18, 35). After the initial recognition reaction between the RNAs, they form a more extensive duplex. Therefore, in the killer systems, the site of initial recognition between the RNAs is coincidental with the nucleation point of duplex formation. Since the duplexes are rapidly cleaved by RNase III, antisense RNA binding confers irreversible mRNA inactivation and decay (25).

The Sok-RNA/hok mRNA-binding mechanism resembles the RNA-IN/RNA-OUT pairing that regulates transposase expression in the mobile genetic element Tn10 (7, 8, 43, 92). In the latter case, the target is the single-stranded 5' end of the tnp mRNA (RNA-IN), which is recognized by a hairpin loop in the antisense RNA, RNA-OUT. Therefore, the situation is the reverse of the Sok-RNA/hok mRNA pairing. Other features are similar: The single-stranded 5' end of one molecule interacts with a loop in the other RNA, and the first contacts are realized with stable G-C base-pairings. According to the mechanism of RNA/RNA duplex initiation and propagation, these systems define a distinct group that is different from the group of RNA pairs that recognize their partner via kissing complex formation (92).

#### MOLECULAR MODEL THAT EXPLAINS PROGRAMMED CELL DEATH

Based on the preceding sections, we can now delineate in molecular detail the series of events that lead to activation of hok translation in plasmid-free cells (visualized in Figure 5). First, a reservoir of the inert full-length hok mRNA accumulates in plasmid-carrying cells. This RNA is continuously processed at its 3' end by the exonucleases, thereby leading to generation of the truncated RNA. After refolding, this RNA contains the antisense RNA target hairpin and therefore is active with respect to translation and antisense RNA binding. In plasmid-carrying cells, Sok-RNA rapidly binds to hok mRNA via the target-loop and thereby prevents its translation. The immediate RNase III cleavage of the duplexed RNAs explains why truncated hok mRNA is not detectable in growing cells (25). Plasmid-free cells that arise by division of plasmid-carrying cells inherit pools of both the inert full-length hok mRNA and the Sok antisense RNA. Due to its instability, the antisense will decay rapidly. Therefore, the continued slow 3' processing of full-length hok mRNA leads to accumulation of the stable truncated mRNA. This, in turn, leads to hok translation and selective killing of the plasmid-free cells. Note that the kinetics of the 3' exonucleolytic processing event is important, since mutations (in the fbi element) that lead to rapid truncation inactivate the PSK mechanism by depleting the reservoir of activable full-length hok mRNA (17, 58).

#### MULTIPLE CHROMOSOME-ENCODED LOCI

A number of chromosome-encoded hok-homologous gene systems have been identified and analyzed (listed in Table 1). The chromosome of *E. coli* K-12 contains five such loci. Even though these loci certainly encode

hok-like killer genes, the proteins are quite distantly related (see Figure 6C). The presence of chromosome-encoded homologues of plasmid-encoded maintenance systems (also including centromere-like partition systems, proteic killer systems, and site-specific recombination systems) is the rule rather than the exception. For example, homologues of the plasmid P1 partition system are present on the chromosomes of *Caulobacter crescentus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (39, 44, 54a, 63). In two cases it has been shown that these systems function to stabilize the inheritance of their chromosomes (39, 54a). Furthermore, two copies of the proteic plasmid stabilization system *pem* of R100 are present on the *E. coli* chromosome (51). However, we find the presence of five hok-like gene systems on the *E. coli* chromosome surprising; it may point to some hitherto uncovered function or feature of this type of gene cassette. The biology of the chromosomal hok-homologous gene systems is briefly described below.

#### HOKA OF *E. COLI* K-12 AND *E. COLI* C

A hok-homologous reading frame located just downstream of the *cspA* locus at 80.1' at the *E. coli* K-12 chromosome was identified by database searching (5, 72). We called the gene *hokA*, and its putative product of 51 aa was denoted HokA. Using **polymerase** chain reaction (PCR), the structural *hokA* gene was cloned into a conditional expression vector. Expression of HokA led to the typical Hok-induced cell-damage response (72). Sequence comparisons showed that an IS150 element is located a few bp upstream of the *hokA* reading frame. Inspection of the nucleotide sequence upstream of the IS-element revealed that the element had transposed into the middle of a gene system similar to the other hok-homologous loci. Thus, the *hokA* locus contains putative *fbi* and *tac* elements. However, sequence alignments also indicated the presence of a 40-bp deletion adjacent to the IS-element, which would inactivate the putative antisense RNA promoter and remove the 5' part of the putative mok-homologous reading frame. Obviously, such a deletion would inactivate the *hokA* locus with respect to the PSK mechanism.

A collection of *E. coli* strains was screened by PCR for the presence of *hokA* loci without an IS150 element. We found that all *E. coli* K-12, K-10, and B strains probably contain *hokA* loci with the IS150 element. However, *E. coli* C appeared to contain a *hokA* locus without the IS150 element. Further cloning and analysis of this locus revealed that it encodes an intact killer gene, a mok-homologous reading-frame, which we denote *mokA*, and an unstable *SokA* antisense RNA of 55 nt (Figure 4). Northern analyses showed that full-length *hokA* mRNA is processed at its 3' end to a stable truncated mRNA (72). The alignment of *hokA* mRNA with the other mRNAs reveals that all major regulatory elements are present, thus indicating that *hokA* of *E. coli* C (and K-12) was derived from an active PSK system (Figure 3). For reasons unknown, *hokA* from *E. coli* C was not clonable in *E. coli* K-12 (72).

#### HOKB OF *E. COLI* K-12

A second hok-homologous reading frame, denoted *hokB*, was found by data base searching, to be located near the *cybB* gene (encoding cytochrome b561) at 32.1' (K Gerdes, unpublished data). Alignment of the putative *hokB* mRNA with the other hok-homologous mRNAs revealed all the regulatory elements as described previously for these mRNAs (Figure 3). The mok-homologous reading frame was denoted *mokB*, and the putative antisense RNA was called *SokB* (Figure 4). The *hokB* locus has not yet been cloned and analyzed.

#### HOKC (GEF) OF *E. COLI* K-12

The *gef* (gene fatale) locus at 0.4' at the *E. coli* K-12 map was identified by Southern analysis using R1 hok-encoding DNA as a hybridization probe (74). It was shown by PCR cloning that *gef*-homologous loci are present in *E. coli* B and C. Furthermore, indirect evidence (i.e. Southern hybridization) suggested that *gef* might be conserved outside of the family of enteric bacteria (74). However, the specificity of these findings remains to be confirmed, and at present there is no direct evidence as to the existence of hok-like genes in Gram-negative bacteria other than the enterics. This interesting supposition awaits further analyses.

The *gef* locus of *E. coli* K-12 was cloned and analyzed (74, 75). A



mok-homologous reading frame (denoted orf69) overlaps with gef and, as in the case of hok, translation of gef was found to be coupled to that of the overlapping reading frame. Furthermore, a trans-acting antisense RNA that regulates translation of orf69 was also identified. The antisense RNA gene was called sof (suppression of fatality). Thus the function of orf69 is similar to that of mok in the hok/sok system. To create a uniform genetic nomenclature, we suggest here that gef, sof, and orf69 be renamed hokC, sokC, and mokC, respectively.

An IS186 element is located 20 nt downstream of the hokC reading frame in *E. coli* K-12 (74). As in the case of hokA, we subtracted the sequence of the IS186 element from the contig encoding hokC. Inspection of the hokC locus revealed the presence of all the structural elements found in hok mRNA (Figure 3). The hokC- and sokC-RNAs are aligned with the other RNAs in Figures 3 and 4, respectively.

#### HOKD (REL<sup>F</sup>) OF *E. COLI* K-12

The *E. coli* relB operon consists of three genes, denoted relB, relE, and relF (2). Mutations in the first gene confer the so-called "delayed relaxed response" upon the host cells (2). By visual inspection of the DNA sequence, the third gene, relF, was identified as a hok-homologous gene (21). To obtain a uniform genetic nomenclature, we suggest that relF be renamed hokD.

The hokD (relF) gene was cloned in a conditional expression vector and shown to encode a protein whose expression elicits the typical Hok-induced damage response (21). Recently, we realized that a putative fbi sequence is located downstream of the hokD gene. However, no putative tac sequence was found upstream of hokD, and the relB locus does not appear to encode an antisense RNA (59). The presence of the fbi sequence suggests that hokD of *E. coli* K-12 is derived from an intact hok-homologous gene system regulated by a mechanism similar to that of the other PSK systems. This finding further suggests that the present-day hokD locus probably constitutes an evolutionary relic of an ancient hok-homologue.

#### HOKE OF *E. COLI* K-12

A fifth hok-homologous reading frame, denoted hokE, was identified by data base searching of the entire *E. coli* K-12 genome. The hokE locus, which is located at 13.1', contains putative tac and fbi regulatory elements, but exhibits irregularities in other elements. Because the meaning of this is not yet clear, we chose not to include the hokE RNAs in the analyses presented here. Curiously, an IS186 element is located just downstream of the hokE reading frame, in a position similar to that of the IS186 element located just downstream of hokC. The HokE protein is quite distantly related to the other Hok-like proteins and is most similar to the SrnB outgroup (see Figure 6).

#### HOKH OF *HAFNIA ALVEI*

A hok-homologous reading frame, denoted hokH, was found by data base searching, located just downstream of the lysine decarboxylase gene of the enteric bacterium *H. alvei* (K Gerdes, unpublished data). Alignment of the putative hokH mRNA with the other mRNAs is shown in Figure 3; it contains all structural elements necessary for the folding pathway (including tac, fbi, metastable hairpin, and the ucb/SDmok target hairpin). However, no mok-homologous reading frame is present in the published sequence. Thus the hokH locus may, as in the cases of the five hok-homologous loci of *E. coli* K-12, constitute an inactivated PSK system. The hokH system has not yet been cloned and analyzed.

#### MECHANISM OF CELL KILLING

##### THE TOXINS

The Hok-like proteins are small membrane-associated polypeptides of [similar]50 aa (28). They consist of two domains: an N-terminal trans-membrane  $\alpha$ -helix, and a C-terminal domain that protrudes into the periplasm (76). The  $\alpha$ -helical domains are hydrophobic, but flanked by positively charged amino acids, thus conforming to the "positive-inside" rule of von Heijne (91). Mutations that change the charged amino acids flanking the hydrophobic domain abolish or reduce the toxicity of the HokC protein (76). The charged amino acids may be required to fix the proteins

in their trans-membrane configuration. The periplasmic domain contains both positively and negatively charged aa, and is thus highly hydrophilic. The Hok-like proteins contain an invariant sequence motif Ala-Tyr-Glu near their C-terminal ends. Mutations that result in changes in this motif suppress or reduce toxicity (76; K Gerdes, unpublished).

#### THE MECHANISM OF CELL KILLING

The Hok-like proteins are very toxic to most Gram-negative species (20, 22, 26, 75, 78) and also to some extent to Gram-positive bacteria (55, 78). Induction of Hok leads to loss of the cell membrane potential, arrest of respiration, efflux of small molecules (i.e. Mg<sup>2+</sup> and ATP), and influx of small extracellular molecules (i.e. ONPG) and even influx of periplasmic proteins such as RNase I (21, 65, 66). By phase contrast microscopy, we observed that the cells change morphology to so-called "ghost-cells" after induction of Hok protein synthesis. These ghost cells are characterized by condensed cell poles and a centrally located clearing, and resemble the ghost cells (empty cell shells) formed after induction of the lysis gene of Fx174 (101). Furthermore, the *srnB* gene of F complements mutations in the *ls* gene (68), which encodes a **holin** (101). Thus the Hok family of proteins bears functional resemblance to this large group of bacteriophage-encoded proteins, collectively known as **holins**. The **holins** create holes or pores in the inner cell membrane, thereby releasing a phage-encoded endolysin to the periplasm, where it degrades the cell wall (101). Thus, all our observations suggest that the Hok-like proteins kill the cells by mediating irreversible damage to the host cell membrane.

Two models have been proposed to explain the cell-killing activity of the Hok-like proteins: The pore model presupposes that an oligomeric form of the toxins forms trans-membrane pore-like structures that permeabilize the membrane. This is similar to the proposed mode of action of the bacteriophage-encoded **holins** (excellently reviewed in References 101, 102). The target model presupposes that the toxins interact with a specific target located in the cell membrane or in the periplasm.

In an effort to identify the cellular target of the Hok-like proteins, HokC-(Gef)--resistant host cells were selected by repeated induction of HokC (77). Two different *E. coli* K-12 host cell lines were used, MC1000 and C600. HokC-resistant cell lines derived from C600 were unstable, meaning that the cells consistently reverted to sensitivity after a number of generations (K Gerdes, unpublished). However, after seven cycles of repeated induction, a stable HokC-resistant derivative of MC1000 was finally obtained and denoted NWL37 (77). The resistant strain tolerates elevated amounts of HokC (Gef), Hok, PndA, and *SrnB'* (77). However, the strain appears sensitive to high amounts of killer protein. Curiously, transduction of the resistance phenotype to strains other than MC1000 resulted in unstable inheritance of the phenotype. These results indicate that stable inheritance of the Hok-resistance phenotype requires additional host mutations, and that MC1000 by default contains these extra mutations. The mutation resulting in stable HokC resistance was mapped to a hitherto uncharacterized reading frame (orf178) located at 55.2' on the *E. coli* map. The resistant host cells contain a conservative change of Asp-64 to Glu in orf178 (77). The putative orf178 gene product exhibits similarity to the RibG proteins from *E. coli* and *Bacillus subtilis*, two enzymes involved in riboflavin biosynthesis (K Gerdes, unpublished observation). The orf178 gene product is not essential since its gene can be deleted without deleterious effects on the host cells. However, orf178 probably does not code for the cellular target of the Hok proteins, since strains deleted of orf178 are sensitive to HokC- (and Hok-) mediated cell killing (77).

#### EVOLUTION OF THE HOK GENE FAMILY

##### PHYLOGENETIC RELATIONSHIPS

The highly conserved structural features of the *hok*-related mRNAs and the toxins encoded by them suggest a common ancestral gene. However, reconstruction of their phylogeny is not straightforward. A simple search for the most parsimonious phylogenetic tree reveals several possible solutions, with similar values of tree lengths (Figure 6). Presumably, this is a consequence of so-called homoplasy (reversions, convergence, and parallel changes) in the sequences that are under two selective pressures:

structural requirements for mRNA folding and coding of functional protein. Therefore the tree based on the alignment of the noncoding regions (Figure 6A) is different from those based on the nucleotide sequences of the coding parts or by the proteins themselves (Figure 6B, C, respectively). A comparison of the tree lengths, calculated for the same regions of RNA sequences, does not give strong preference to any of the trees. In the noncoding region, the most parsimonious tree (Figure 6A) contains 347 nucleotide substitutions, whereas both the other trees have 379 changes. In the coding part, the situation is reversed. The trees shown in Figure 6A, B, and C have 374, 340, and 346 substitutions, respectively [note that *hokD* (*relF*) was also included]. Thus being applied for the entire mRNAs, all three trees have comparable lengths. It remains to be determined which of the three is the correct one. However, this determination requires more complicated approaches that take into account assumptions on homoplasy and nonequal substitution rates in RNA structures (37, 90).

Even though the precise phylogenetic relationships in the *hok* gene family are not clear, some conclusions can be drawn. All three trees separate *srnB* as an outgroup, whereas none of them distinguishes chromosomal genes in a separate group distinct from the plasmid-encoded systems. Similarly, *hokH* from *H. alvei* does not form an outgroup. It is noteworthy that the two systems from *F* (*flm* and *srnB*) are quite diverse (Figure 6). All known *pnd* genes are closely related, and two of the chromosomal genes from *E. coli* K-12, *hokC* (*gef*), and *hokD* (*relF*), form a subgroup.

#### FUNCTION OF THE PLASMID-ENCODED PSK SYSTEMS

Compared to the apparent diversity at the level of primary structure, the strong conservation of the structural elements is very surprising. In the plasmid-encoded gene systems, the conservation is a prerequisite for the functioning of the PSK mechanism. An interesting question arises why such "selfish" behavior of plasmids is so strongly conserved, and whether these genes are really "selfish." The problem is even more intriguing because other bacterial systems that mediate programmed cell death share the main properties with the antisense RNA-regulated gene systems. In the proteic plasmid stabilization systems, the mechanism also involves a stable toxin but a labile protein antidote (reviewed in Reference 42). Recent reports demonstrate that well-known restriction modification systems can mediate plasmid stabilization by PSK. In the latter case, the antidote is a DNA methylase that prevents the lethal DNA digestion by the corresponding restriction enzyme. The PSK phenomenon is triggered by dilution of the methylase in plasmid-free cells, which allows the restriction enzyme to degrade the cellular DNA (46, 56). Such diverse ways of plasmid "selfishness," which makes the bacterial cells addicted to plasmid presence, suggest that the plasmid-bacterium pair may be considered as a symbiotic parasite-host couple with mutual benefits (for discussion, see Reference 100). For the plasmid, the main benefit is obvious: It "enslaves" the host to reproduce it. For the host, the advantage of bearing a plasmid could be an increased evolutionary potential. Also, stabilization of the plasmid may be necessary if the plasmid is advantageous in some stress conditions only, but normally is a burden for the cell.

#### FUNCTION OF THE CHROMOSOME-ENCODED PSK SYSTEMS

The presence of numerous *hok*-homologues on the bacterial chromosome suggests that these genes themselves could be beneficial. The list in Table 1 shows that all plasmid-encoded systems are active with respect to PSK, and that none of the chromosome-encoded systems has this property. It is difficult to imagine that bacterial chromosomes with their compact genetic information carry numerous gene systems without present or past function. Despite considerable effort, we have not yet identified *hok*-homologous systems that are active with respect to PSK from a chromosome. We thus favor the idea that the chromosomes recruited the PSK systems from conjugative low-copy-number plasmids, and that the systems were inactivated when resident on their present-day chromosome. In our view, this leaves open the possibility that active chromosome-encoded PSK systems may, under certain conditions, specify a beneficial function. If this function is connected with cell killing, then their rapid inactivation would be selected for and thus would explain the lack of PSK activity. One

possibility is that PSK systems could provoke altruistic suicide of the major fraction of a bacterial population. Survivors from such events would have an increased probability of carrying an inactivated PSK system. Recently, evidence was obtained that the *hok/sok* system mediates exclusion of bacteriophage T4 (71). The exclusion phenotype may be related to the PSK mechanism in the following way: Infection by the phage leads to a rapid cessation of host cell transcription and a switch to phage mRNA synthesis. This, in turn, leads to depletion of the *Sok*-RNA pool and subsequent activation of the PSK mechanism. If killing occurs before mature phages have developed then such altruistic suicide would prevent or reduce the spread of the virus in the bacterial population. It is not known if the PSK systems elicit such a response in natural populations, but the exclusion was not very efficient, even when the *hok/sok* system was present on a high-copy-number plasmid (71). However, these observations do not explain the fact that all known plasmid-encoded systems are active and that all chromosome-encoded systems are inactive with respect to PSK. Thus, the function of the chromosome-encoded systems remains an enigma that has to be addressed experimentally.

#### HOK-LIKE PROTEINS IN MITOCHONDRIA

In searching for a mechanism that could secure stable inheritance of mitochondrial genomes, it was observed serendipitously that the A8 subunits of mitochondrial ATP synthases exhibit weak similarity to the *Hok*-like proteins (40). It was argued that these essential components of the ATP synthases may have evolved from an ancestral PSK system into the present-day proteins. Although the hydropathy profiles of the *Hok*-like proteins and the A8 subunits are strikingly similar, the homologies at the level of primary sequences are virtually insignificant (40). Therefore the similarity between these two groups of membrane proteins could also have arisen by convergent evolution.

#### A KILLER GENE SYSTEM IN GRAM-POSITIVE BACTERIA

The conjugative low-copy-number plasmid *pAD1* from *Enterococcus faecalis* is stably maintained because of a region termed *par* (94). The *par* locus encodes two small RNAs, RNA I and II, of 210 and 65 nt, respectively. The RNAs are convergently transcribed, and the major part of RNA II is complementary to the 3' end of RNA I (93). Expression of RNA I without RNA II is lethal to the host cells. Therefore, RNA II seems to be an antisense RNA that negatively regulates the function of RNA I. These and other observations prompted Weaver et al to suggest that *par* specifies a PSK system in which RNA I encodes a toxic function and that the toxicity is counteracted by RNA II. If this model holds, then the *par* system of *pAD1* is the first example of a PSK system from Gram-positive bacteria.

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#### Added material

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Table 1 Plasmid- and chromosome-encoded *hok*-homologous loci with known DNA sequence

#### FOOTNOTE

a nd: not determined;

b tac denotes the translational activator elements at the mRNA 5'

ends, and fbi denotes the fold-back inhibitory sequences at the mRNA 5' ends, see the text;

c A putative fbi element is located downstream of hokD (relF).

Figure 1 A: Structural organization and regulatory elements of the hok/sok system of plasmid R1. Genetic nomenclature: mok, modulation of killing; hok, host killing gene; sok, suppression of killing; sokT, Sok-RNA target region; fbi, fold-back-inhibition element; tac, translational activation element. B: Secondary structure of Sok antisense RNA from plasmid R1. The RNase E cleavage site at the RNA 5' end is indicated. Non-Watson-Crick G-U base-pairings are shown with dots.

Figure 2 Folding pathway of hok mRNA. A: early steps in the folding of the nascent transcript showing the sequestration of the mok and hok Shine-Dalgarno regions. An M indicates the 5' metastable structure; ucb, upstream complementary box; dcb, downstream complementary box. B: Secondary structure of full-length hok mRNA showing the fbi-tac interaction, the ucb/SDmok and dcb/SDhok interactions, and the upper part of the tac-stem. C: Secondary structure of the truncated, refolded hok mRNA. The ucb/SDmok interaction is disrupted by the formation of the stable tac-stem. In turn, this leads to the formation of the antisense RNA target stem-loop containing the SDmok/dcb interaction. Arrow denoted sokT indicates the nucleotide complementary to the very first nucleotide of the 5' end of Sok-RNA and thus marks the 3' border of the Sok target region (sokT) in hok mRNA. The folding pathway of hok mRNA was from (18) and (35).

Figure 3 Alignment of plasmid- and chromosome-encoded hok-homologous mRNAs. Covarying nucleotides are shown in color. Color codes: red: all coupled covariations (bases that covary with more than one partner, see the text); green: covariations in tac and target stems; blue: covariations in ucb/SDmok and dcb/SDhok stems. Secondary structure elements in hok mRNA are indicated by colored lines above the sequences. The short arrow pointing leftward marks the nucleotides complementary to the very 5' nucleotide of the antisense RNAs. This base position in the alignment is an invariant rC. The AUG start-codon of hok, SDmok, and SDhok are indicated with dark blue lines above the sequences. Deletions are shown by dashes, and the coding parts downstream of the AUG start codons of the killer genes are symbolized by dots. Invariant nucleotides are indicated by asterisks below the sequences.

Figure 4 Alignment of the antisense RNAs encoded by the hok-homologous gene systems. Nucleotide covariations are shown in bold. The single-stranded 5' ends and the loop regions are underlined twice, and invariant nucleotides are indicated by asterisks below the sequences. The bases complementary to the mok and mok-homologous start-codons are italicized. Dashes were introduced to optimize the alignment. SokA was from *E. Coli* C, whereas SokB and SokC (formerly Sof) were from *E. Coli* K-12.

Figure 5 Model to explain induction of hok translation in plasmid-free cells. During steady-state growth, full-length hok mRNA accumulates. Because of its folded structure, hok mRNA does not bind the antisense RNA (the primary Sok-RNA target, sokT, is shielded due to the fold-back structure), and the mRNA is not translated due to the ucb/SDmok interaction. The full-length hok mRNA is processed at its 3' end in both plasmid-carrying and plasmid-free cells. The processing triggers a refolding of the mRNA into a configuration that allows translation and antisense RNA binding. A: In plasmid-carrying cells, which contain Sok-RNA, the truncated mRNA is rapidly bound by the antisense RNA. This leads to RNA duplex formation and RNase III cleavage. B: In plasmid-free cells, in which Sok-RNA has decayed, the truncated refolded mRNA accumulates. This leads to translation and killing of the plasmid-free cells ensues. Genetic symbols as in Figure 1.

Figure 6 Three alternative evolutionary trees of the hok gene family based on parsimony analysis for different regions of the mRNAs. A: Tree based on the noncoding RNA sequences; B: tree based on the coding RNA sequences; C: tree based on the protein sequences.

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--metabolism--ME; Chlorides--pharmacology--PD; DNA Primers--genetics--GE;  
Dipeptidyl Peptidases--genetics--GE; Escherichia coli--genetics--GE; Food  
Microbiology; Food Technology; Gene Expression--drug effects--DE; Gene  
Fusion; Genetic Markers; Lactococcus lactis--drug effects--DE; Lactococcus  
lactis--metabolism--ME; Osmotic Fragility; **Polymerase** Chain Reaction;  
Promoter Regions (Genetics)

CAS Registry No.: 0 (Chlorides); 0 (DNA Primers); 0 (Genetic  
Markers)

Enzyme No.: EC 3.4.14.- (Dipeptidyl Peptidases); EC 3.4.14.- (PepX  
dipeptidyl aminopeptidase)

Record Date Created: 19980213

induction, corresponding to an approximately 100-fold increase over the normal lethal level of **holin**. Characterization of this expression system is presented and discussed with respect to the current model of **holin** function.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: \*Bacteriophage lambda--pathogenicity--PY; \*Membrane Proteins--biosynthesis--BI; \*Viral Proteins--biosynthesis--BI; Amino Acid Sequence; Bacteriophage lambda--genetics--GE; Bacteriophage lambda--metabolism--ME; Base Sequence; Cell Membrane Permeability; DNA, Viral--metabolism--ME; Electrophoresis, Polyacrylamide Gel; Endopeptidases--metabolism--ME; Escherichia coli; Gene Expression; Membrane Proteins--genetics--GE; Molecular Sequence Data; Viral Proteins--genetics--GE

CAS Registry No.: 0 (DNA, Viral); 0 (Membrane Proteins); 0 (S holin, bacteriophage lambda); 0 (Viral Proteins); 0 (bacteriophage lambda lysis effector protein S105); 0 (bacteriophage lambda lysis inhibitor protein S107)

Enzyme No.: EC 3.4.- (Endopeptidases); EC 3.4.99.- (endolysin)

Record Date Created: 19980630

32/9/19 (Item 19 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

10337725 99328986 PMID: 10400598

**Lysis and lysis inhibition in bacteriophage T4: rV mutations reside in the holin t gene.**

Dressman H K; Drake J W

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.

Journal of bacteriology (UNITED STATES) Jul 1999, 181 (14) p4391-6, ISSN 0021-9193 Journal Code: 2985120R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Upon infecting populations of susceptible host cells, T-even bacteriophages maximize their yield by switching from lysis at about 25 to 35 min at 37 degrees C after infection by a single phage particle to long-delayed lysis (lysis inhibition) under conditions of sequential infection occurring when free phages outnumber host cells. The timing of lysis depends upon gene t and upon one or more rapid-lysis (r) genes whose inactivation prevents lysis inhibition. t encodes a **holin** that mediates the movement of the T4 endolysin through the inner cell membrane to its target, the cell wall. The rI protein has been proposed to sense superinfection. Of the five reasonably well characterized r genes, only two, rI and rV, are clearly obligatory for lysis inhibition. We show here that rV mutations are alleles of t that probably render the t protein unable to respond to the lysis inhibition signal. The tr alleles cluster in the 5' third of t and produce a strong r phenotype, whereas conditional-lethal t alleles produce the classical t phenotype (inability to lyse) and other t alleles produce additional, still poorly understood phenotypes. tr mutations are dominant to t+, a result that suggests specific ways to probe T4 **holin** function.

Descriptors: \*Bacteriophage T4--genetics--GE; \*Escherichia coli--virology--VI; \*Gene Expression Regulation, Viral; \*Genes, Viral; \*Lysogeny--genetics--GE; \*Viral Proteins--genetics--GE; Amino Acid Sequence; Bacteriophage T4--physiology--PH; DNA, Viral--analysis--AN; Molecular Sequence Data; Polymerase Chain Reaction--methods--MT; Sequence Analysis, DNA; Viral Proteins--chemistry--CH

CAS Registry No.: 0 (DNA, Viral); 0 (Viral Proteins); 0 (t holin, bacteriophage T4)

Record Date Created: 19990802

32/9/21 (Item 21 from file: 98)

DIALOG(R) File 98:General Sci Abs/Full-Text

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#### DESCRIPTORS:

Plasmids; Antisense genetics; Molecular evolution; Apoptosis

32/9/36 (Item 36 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

10343749 99350405 PMID: 10419939

**Evidence for a holin -like protein gene fully embedded out of frame in the endolysin gene of *Staphylococcus aureus* bacteriophage 187.**

Loessner M J; Gaeng S; Scherer S

Institut fur Mikrobiologie, Forschungszentrum fur Milch und Lebensmittel Weihenstephan, Technische Universitat Munchen, D-85350 Freising, Germany.

Journal of bacteriology (UNITED STATES) Aug 1999, 181 (15) p4452-60, ISSN 0021-9193 Journal Code: 2985120R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

We have cloned, sequenced, and characterized the genes encoding the lytic system of the unique *Staphylococcus aureus* phage 187. The endolysin gene *ply187* encodes a large cell wall-lytic enzyme (71.6 kDa). The catalytic site, responsible for the hydrolysis of staphylococcal peptidoglycan, was mapped to the N-terminal domain of the protein by the expression of defined *ply187* domains. This enzymatically active N terminus showed convincing amino acid sequence homology to an N-acetylmuramoyl-L-alanine amidase, whereas the C-terminal part, whose function is unknown, revealed striking relatedness to major staphylococcal autolysins. An additional reading frame was identified entirely embedded out of frame (+1) within the 5' region of *ply187* and was shown to encode a small, hydrophobic protein of **holin** -like function. The *hol187* gene features a dual-start motif, possibly enabling the synthesis of two products of different lengths (57 and 55 amino acids, respectively). Overproduction of *Hol187* in *Escherichia coli* resulted in growth retardation, leakiness of the cytoplasmic membrane, and loss of de novo ATP synthesis. Compared to other **holins** identified to date, *Hol187* completely lacks the highly charged C terminus. The secondary structure of the polypeptide is predicted to consist of two small, antiparallel, hydrophobic, transmembrane helices. These are supposed to be essential for integration into the membrane, since site-specific introduction of negatively charged amino acids into the first transmembrane domain (V7D G8D) completely abolished the function of the *Hol187* polypeptide. With antibodies raised against a synthetic 18-mer peptide representing a central

part of the protein, it was possible to detect Hol187 in the cytoplasmic membrane of phage-infected *S. aureus* cells. An important indication that the protein actually functions as a **holin** in vivo was that the gene (but not the V7D G8D mutation) was able to complement a phage lambda Sam mutation in a nonsuppressing *E. coli* HB101 background. Plaque formation by *lambda*gt11::hol187 indicated that both phage genes have analogous functions. The data presented here indicate that a putative **holin** is encoded on a different reading frame within the enzymatically active domain of ply187 and that the **holin** is synthesized during the late stage of phage infection and found in the cytoplasmic membrane, where it causes membrane lesions which are thought to enable access of Ply187 to the peptidoglycan of phage-infected *Staphylococcus* cells.

Descriptors: \*Endopeptidases--genetics--GE; \*Membrane Proteins--genetics--GE; \*Staphylococcus Phages--genetics--GE; \*Staphylococcus aureus--virology--VI; Amino Acid Sequence; Base Sequence; Cloning, Molecular; DNA, Viral--genetics--GE; *Escherichia coli*; Kinetics; Membrane Proteins--chemistry--CH; Membrane Proteins--metabolism--ME; Molecular Sequence Data; **Polymerase** Chain Reaction; Protein Conformation; Recombinant Fusion Proteins--biosynthesis--BI; Recombinant Fusion Proteins--chemistry--CH; Recombinant Fusion Proteins--metabolism--ME; Sequence Alignment; Sequence Homology, Amino Acid; *Staphylococcus* Phages--enzymology--EN; *Staphylococcus* Phages--physiology--PH; beta-Galactosidase--genetics--GE  
Molecular Sequence Databank No.: GENBANK/Y07740  
CAS Registry No.: 0 (DNA, Viral); 0 (Hol187 protein); 0 (Membrane Proteins); 0 (Recombinant Fusion Proteins)  
Enzyme No.: EC 3.2.1.23 (beta-Galactosidase); EC 3.4.-- (Endopeptidases); EC 3.4.99.-- (endolysin)  
Record Date Created: 19990819

32/9/40 (Item 40 from file: 156)

DIALOG(R)File 156:ToxFile

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01230624 98069482 PMID: 9406408

**A chloride-inducible gene expression cassette and its use in induced lysis of *Lactococcus lactis*.**

Sanders J W; Venema G; Kok J

Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Haren, The Netherlands.

Applied and environmental microbiology (UNITED STATES) Dec 1997, 63 (12) p4877-82, ISSN 0099-2240 Journal Code: 7605801

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: Toxbib ; INDEX MEDICUS

A chloride-inducible promoter previously isolated from the chromosome of *Lactococcus lactis* (J. W. Sanders, G. Venema, J. Kok, and K. Leenhouts, *Mol. Gen. Genet.*, in press) was exploited for the inducible expression of homologous and heterologous genes. An expression cassette consisting of the positive-regulator gene *gadR*, the chloride-inducible promoter *P<sub>gad</sub>*, and the translation initiation signals of *gadC* was amplified by PCR. The cassette was cloned upstream of *Escherichia coli lacZ*, the **holin**-lysin cassette (*lytPR*) of the lactococcal bacteriophage *rlt*, and the autolysin gene of *L. lactis*, *acmA*. Basal activity of *P<sub>gad</sub>* resulted in a low level of expression of all three proteins. Growth in the presence of 0.5 M NaCl of a strain containing the *gadC::lacZ* fusion resulted in a 1,500-fold increase of beta-galactosidase activity. The background activity levels of *LytPR* and *AcmA* had no deleterious effects on cell growth, but induction of lysin expression by addition of 0.5 M NaCl resulted in inhibition of growth. Lysis was monitored by following the release of the cytoplasmic marker enzyme *PepX*. Released *PepX* activity was maximal at 1 day after induction of *lytPR* expression with 0.1 M NaCl. Induction of *acmA* expression resulted in slower release of *PepX* from the cells. The presence of the inducing agent NaCl resulted in the stabilization of osmotically fragile cells.

Tags: Support, Non-U.S. Gov't

Descriptors: \*Gene Expression; \*Genes, Bacterial; \**Lactococcus lactis*--genetics--GE; Bacteriophages--genetics--GE; Base Sequence; Cell Wall

9667152 98069482 PMID: 9406408

**A chloride-inducible gene expression cassette and its use in induced lysis of *Lactococcus lactis*.**

Sanders J W; Venema G; Kok J

Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Haren, The Netherlands.

Applied and environmental microbiology (UNITED STATES) Dec 1997, 63 (12) p4877-82, ISSN 0099-2240 Journal Code: 7605801

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A chloride-inducible promoter previously isolated from the chromosome of *Lactococcus lactis* (J. W. Sanders, G. Venema, J. Kok, and K. Leenhouts, Mol. Gen. Genet., in press) was exploited for the inducible expression of homologous and heterologous genes. An expression cassette consisting of the positive-regulator gene *gadR*, the chloride-inducible promoter *P<sub>gad</sub>*, and the translation initiation signals of *gadC* was amplified by PCR. The cassette was cloned upstream of *Escherichia coli lacZ*, the *holin*-lysin cassette (*lytPR*) of the lactococcal bacteriophage *rlt*, and the autolysin gene of *L. lactis*, *acmA*. Basal activity of *P<sub>gad</sub>* resulted in a low level of expression of all three proteins. Growth in the presence of 0.5 M NaCl of a strain containing the *gadC::lacZ* fusion resulted in a 1,500-fold increase of beta-galactosidase activity. The background activity levels of *LytPR* and *Acma* had no deleterious effects on cell growth, but induction of lysin expression by addition of 0.5 M NaCl resulted in inhibition of growth. Lysis was monitored by following the release of the cytoplasmic marker enzyme *PepX*. Released *PepX* activity was maximal at 1 day after induction of *lytPR* expression with 0.1 M NaCl. Induction of *acmA* expression resulted in slower release of *PepX* from the cells. The presence of the inducing agent NaCl resulted in the stabilization of osmotically fragile cells.

Tags: Support, Non-U.S. Gov't

Descriptors: \*Gene Expression; \*Genes, Bacterial; \**Lactococcus lactis* --genetics--GE; Bacteriophages--genetics--GE; Base Sequence; Cell Wall --metabolism--ME; Chlorides--pharmacology--PD; DNA Primers--genetics--GE; Dipeptidyl Peptidases--genetics--GE; *Escherichia coli*--genetics--GE; Food Microbiology; Food Technology; Gene Expression--drug effects--DE; Gene Fusion; Genetic Markers; *Lactococcus lactis*--drug effects--DE; *Lactococcus lactis*--metabolism--ME; Osmotic Fragility; **Polymerase** Chain Reaction; Promoter Regions (Genetics)

CAS Registry No.: 0 (Chlorides); 0 (DNA Primers); 0 (Genetic Markers)

Enzyme No.: EC 3.4.14.- (Dipeptidyl Peptidases); EC 3.4.14.- (*PepX* dipeptidyl aminopeptidase)

Record Date Created: 19980213

33/9/9 (Item 3 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

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04575992 Genuine Article#: TU511 Number of References: 58

**Title: GENETIC-EVIDENCE FOR AN ACTIVATOR REQUIRED FOR INDUCTION OF COLICIN-LIKE BACTERIOCIN 28B PRODUCTION IN *SERRATIA-MARCESCENS* BY DNA-DAMAGING AGENTS**

Author(s): FERRER S; VIEJO MB; GUASCH JF; ENFEDAQUE J; REGUE M

Corporate Source: UNIV BARCELONA,FAC PHARM,DIV HLTH SCI,DEPT MICROBIOL & PARASITOL/E-08028 BARCELONA//SPAIN/; UNIV BARCELONA,FAC PHARM,DIV HLTH SCI,DEPT MICROBIOL & PARASITOL/E-08028 BARCELONA//SPAIN/

Journal: JOURNAL OF BACTERIOLOGY, 1996, V178, N4 (FEB), P951-960

ISSN: 0021-9193

Language: ENGLISH Document Type: ARTICLE

Geographic Location: SPAIN

Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences

Journal Subject Category: MICROBIOLOGY

**Abstract:** Bacteriocin 28b production is induced by mitomycin in wild-type *Serratia marcescens* 2170 but not in *Escherichia coli* harboring the bacteriocin 28b structural gene (*bss*). Studies with a *bss-lacZ*

transcriptional fusion showed that mitomycin increased the level of bss gene transcription in *S. marcescens* but not in the *E. coli* background, A *S. marcescens* Tn5 insertion mutant was obtained (*S. marcescens* 2170 reg::Tn5) whose bacteriocin 28b production and bss gene transcription were not increased by mitomycin treatment, Cloning and DNA sequencing of the mutated region showed that the Tn5 insertion was flanked by an SOS box sequence and three genes that are probably cotranscribed (regA, regB, and regC), These three genes had homology to phage holins, phage lysozymes, and the Ogr transcriptional activator of P2 and related bacteriophages, respectively, Recombinant plasmid containing this wild-type DNA region complemented the reg::TnS regulatory mutant, A transcriptional fusion between a 157-bp DNA fragment, containing the apparent SOS box upstream of the regA gene, and the caf gene showed increased chloramphenicol acetyltransferase activity upon mitomycin treatment, Upstream of the bss gene, a sequence similar to the consensus sequence proposed to bind Ogr protein was found, but no sequence similar to an SOS box was detected. Our results suggest that transcriptional induction of bacteriocin 28b upon mitomycin treatment is mediated by the regC gene whose own transcription would be LexA dependent.

Identifiers--KeyWords Plus: ESCHERICHIA-COLI; NUCLEOTIDE-SEQUENCE; SATELLITE BACTERIOPHAGE-P4; EXTRACELLULAR PROTEINS; LATE PROMOTER; EXPRESSION; CLONING; REGION; RECA; CLOACIN-DF13

Research Fronts: 94-4806 002 (GENE ORGANIZATION; LONG-CHAIN FATTY-ACID TRANSPORT; TRANSCRIPTION FACTOR)

94-0736 001 (MURINE CYTOMEGALOVIRUS GENE; HUMAN PROTEIN SEQUENCES; IDENTIFICATION OF ROFA)

94-1703 001 (DNA DAMAGE-INDUCIBLE REPLICATION OF THE ESCHERICHIA-COLI CHROMOSOME; RECA GENE; UV INDUCTION)

94-6345 001 (ESCHERICHIA-COLI RNA- **POLYMERASE** ; LACUV5 PROMOTER; TRANSCRIPTION INITIATION; EXPRESSION ANALYSIS)

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YOUNG RY, 1992, V56, P430, MICROBIOL REV

33/9/10 (Item 4 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2002 Inst for Sci Info. All rts. reserv.

03428067 Genuine Article#: PE570 Number of References: 64

**Title: CONTROLLED EXPRESSION AND STRUCTURAL ORGANIZATION OF A  
LACTOCOCCUS-LACTIS BACTERIOPHAGE LYSIN ENCODED BY 2 OVERLAPPING GENES**

Author(s): SHEARMAN CA; JURY KL; GASSON MJ

Corporate Source: AFRC, INST FOOD RES, NORWICH RES PK/NORWICH NR4  
7UA/NORFOLK/ENGLAND/

Journal: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 1994, V60, N9 (SEP), P  
3063-3073

ISSN: 0099-2240

Language: ENGLISH Document Type: ARTICLE

Geographic Location: ENGLAND

Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences; CC AGRI--  
Current Contents, Agriculture, Biology & Environmental Sciences

Journal Subject Category: BIOTECHNOLOGY & APPLIED MICROBIOLOGY

**Abstract:** The phi vML3 bacteriophage lysin is specific for lactococci and could be used to promote enzyme release during cheese manufacture. The level of lysin expression from the cloned gene using its own upstream sequences is very low. Expression in *Escherichia coli* by using a synthetic hybrid lysin gene and a series of BAL 31 deletions of the original cloned DNA fragment suggested that the start of the gene had previously been incorrectly assigned. Reevaluation of homology between the lysin and *Bacillus subtilis* PZA protein 15 led to the identification of a new potential ribosome binding site (RBS). A 0.72-kb PCR-generated fragment including this RBS and the complete lysin gene was expressed and inducibly controlled. The translational start of the lysin gene was identified as an isoleucine codon, and this may lead to a low translation rate. During the analysis of the BAL 31 deletion fragments, two proteins of 20 and 8 kDa were shown to be expressed from the originally defined lysin gene. The DNA sequence has a second open reading frame with a good RBS and two potential start methionines. The smaller lysin protein was isolated, and the N terminus was sequenced, confirming that one methionine codon acted as the start of a second gene. The larger lysin protein has homology with lysozymes. The smaller lysin protein has some features resembling those of a *holin*. The possible roles of these two proteins in lysis of lactococci are discussed.

Identifiers--KeyWords Plus: DUAL-START MOTIF; LAMBDA-S GENE;  
ESCHERICHIA-COLI; PHAGE PHI-29; NUCLEOTIDE-SEQUENCES; HOMOLOGOUS  
SEQUENCE; SECONDARY-STRUCTURE; PLASMID VECTORS; CHEDDAR CHEESE; RNA-  
POLYMERASE

Research Fronts: 92-0869 002 (LACTOCOCCUS-LACTIS SUBSP LACTIS;  
BACTERIOPHAGE RESISTANCE; BACTERIOCIN PLASMID)



- 92-3056 002 (UPTAKE OF SURFACTANT PROTEIN-B; CASEIN KINASE-II;  
CATALYTIC SUBUNITS)
- 92-3995 002 (PROTEIN SECONDARY STRUCTURE; FUNCTIONAL TOPOGENIC DOMAINS;  
ALPHA-HELIX PREDICTION)
- 92-4812 002 (PUTATIVE ANAEROBIC COPROPORPHYRINOGEN-III OXIDASE IN  
RHODOBACTER-SPHAEROIDES; TRANSCRIPTIONAL REGULATORY ELEMENT; FUNCTIONAL  
EXPRESSION)
- 92-8077 001 (EXPRESSION OF A RECOMBINANT GENE; VIRAL ASSEMBLY PROTEIN;  
VACCINIA VIRUS VECTORS; DNA-BINDING INVITRO; XENOPUS OOCYTES; DIFFERENT  
EXTRACELLULAR DOMAINS)

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09750839 98175413 PMID: 9515662

**Bacteriophages show promise as antimicrobial agents.**

**Alisky J** ; Iczkowski K; Rapoport A; Troitsky N

Department of Community and Family Medicine, St. Louis University School of Medicine, MO 63014, USA.

Journal of infection (ENGLAND) Jan 1998 , 36 (1) p5-15, ISSN 0163-4453 Journal Code: 7908424

Document type: Journal Article; Review; Review Literature

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The emergence of antibiotic-resistant bacteria has prompted interest in alternatives to conventional drugs. One possible option is to use bacteriophages (phage) as antimicrobial agents. We have conducted a literature review of all Medline citations from 1966-1996 that dealt with the therapeutic use of phage. There were 27 papers from Poland, the Soviet Union, Britain and the U.S.A. The Polish and Soviets administered phage orally, topically or systemically to treat a wide variety of antibiotic-resistant pathogens in both adults and children. Infections included suppurative wound infections, gastroenteritis, sepsis, osteomyelitis, dermatitis, empyemas and pneumonia; pathogens included Staphylococcus, Streptococcus, Klebsiella, Escherichia, Proteus, Pseudomonas, Shigella and Salmonella spp. Overall, the Polish and Soviets reported success rates of 80-95% for phage therapy, with rare, reversible gastrointestinal or allergic side effects. However, efficacy of phage was determined almost exclusively by qualitative clinical assessment of patients, and details of dosages and clinical criteria were very sketchy. There were also six British reports describing controlled trials of phage in animal models (mice, guinea pigs and livestock), measuring survival rates and other objective criteria. All of the British studies raised phage against specific pathogens then used to create experimental infections. Demonstrable efficacy against Escherichia, Acinetobacter, Pseudomonas and Staphylococcus spp. was noted in these model systems. Two U.S. papers dealt with improving the bioavailability of phage. Phage is sequestered in the spleen and removed from circulation. This can be overcome by serial passage of phage through mice to isolate mutants that resist sequestration. In conclusion, bacteriophages may show promise for treating antibiotic resistant pathogens. To facilitate further progress, directions for future research are discussed and a directory of authors from the reviewed papers is provided. (62 Refs.)

Tags: Animal; Human

Descriptors: \*Bacterial Infections--therapy--TH; \*Bacteriophages --physiology--PH; Bacteriolysis; Drug Resistance, Microbial; Mice

Record Date Created: 19980428

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# NiceProt View of SWISS-PROT: P50029

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[\[Keywords\]](#) [\[Features\]](#) [\[Sequence\]](#) [\[Tools\]](#)

General information about the entry

Entry name	DP3B_STAAM
Primary accession number	P50029
Secondary accession numbers	None
Entered in SWISS-PROT in	Release 34, October 1996
Sequence was last modified in	Release 34, October 1996
Annotations were last modified in	Release 41, June 2002
<b>Name and origin of the protein</b>	
Protein name	DNA polymerase III, beta chain
Synonym	EC <u>2.7.7.7</u>
Gene name	DNAN or <u>SAV0002</u> or <u>SA0002</u> or <u>MW0002</u>
From	<u>Staphylococcus aureus</u> [TaxID: <u>(strain Mu50 / ATCC</u> <u>158878]</u> <u>700699)</u> <u>Staphylococcus aureus</u> [TaxID: <u>(strain N315)</u> <u>158879]</u> <u>Staphylococcus aureus</u> [TaxID: <u>(strain MW2)</u> <u>196620]</u> <u>Staphylococcus aureus</u> [TaxID: <u>1280]</u>
Taxonomy	<u>Bacteria</u> ; <u>Firmicutes</u> ; <u>Bacillales</u> ; <u>Staphylococcus</u> .

## References

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**STRAIN**=Mu50 / ATCC 700699, and N315;  
**MEDLINE**=21311952; PubMed=11418146; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
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**STRAIN**=MW2;  
**MEDLINE**=22040717; PubMed=12044378; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Baba T.](#), [Takeuchi F.](#), [Kuroda M.](#), [Yuzawa H.](#), [Aoki K.-I.](#), [Oguchi A.](#), [Nagai Y.](#),  
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### Comments

**FUNCTION:** DNA POLYMERASE III IS A COMPLEX, MULTICHAIN ENZYME RESPONSIBLE FOR MOST OF THE REPLICATIVE SYNTHESIS IN BACTERIA. THIS DNA POLYMERASE ALSO EXHIBITS 3' TO 5' EXONUCLEASE ACTIVITY. THE BETA CHAIN IS REQUIRED

FOR INITIATION OF REPLICATION ONCE IT IS CLAMPED ONTO DNA, IT SLIDES FREELY (BIDIRECTIONAL AND ATP-INDEPENDENT) ALONG DUPLEX DNA (*BY SIMILARITY*).

**CATALYTIC ACTIVITY:** N deoxynucleoside triphosphate = N diphosphate + {DNA}<sub>N</sub>.

**SUBUNIT:** CONTAINS A CORE (COMPOSED OF ALPHA, EPSILON, AND THETA CHAINS) THAT CAN REPAIR SHORT GAPS CREATED BY NUCLEASE IN DUPLEX DNA. FOR EFFICIENT REPLICATION OF THE LONG, SINGLE-STRANDED TEMPLATES, POL III REQUIRES THE AUXILIARY CHAINS BETA, GAMMA, AND DELTA (*BY SIMILARITY*).

**SUBCELLULAR LOCATION:** Cytoplasmic (*By similarity*).

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### Cross-references

EMBL	AP003358;	[ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ]
	BAB56164.1; -.	[ <a href="#">CoDingSequence</a> ]
	AP003129; BAB41218.1;	[ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ]
	-.	[ <a href="#">CoDingSequence</a> ]
	AP004822;	[ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ]
	BAB93867.1; -.	[ <a href="#">CoDingSequence</a> ]
CMR	P50029; SAV0002.	
InterPro	<a href="#">IPR001001</a> ; DNA_polIII_beta. <a href="#">Graphical view of domain structure.</a>	
Pfam	<a href="#">PF00712</a> ; DNA_pol3_beta; 1.	
	<a href="#">PF02767</a> ; DNA_pol3_beta_2; 1.	
	<a href="#">PF02768</a> ; DNA_pol3_beta_3; 1.	
SMART	<a href="#">SM00480</a> ; POL3Bc; 1.	
TIGRFAMs	<a href="#">TIGR00663</a> ; dnan; 1.	
ProDom	[ <a href="#">Domain structure</a> / <a href="#">List of seq. sharing at least 1 domain</a> ].	
BLOCKS	<a href="#">P50029</a> .	
ProtoNet	<a href="#">P50029</a> .	
ProtoMap	<a href="#">P50029</a> .	
PRESAGE	<a href="#">P50029</a> .	
DIP	<a href="#">P50029</a> .	
ModBase	<a href="#">P50029</a> .	
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## Keywords

Transferase; DNA-directed DNA polymerase; DNA replication;  
Complete proteome.

## Features

None

## Sequence information

Length: 377 AA	Molecular weight: 41913 Da	CRC64: 0A985EF94E044FBC [This is a checksum on the sequence]
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MMEFTIKRDY FITQLNDTLK AISPRTTLPI LTGIKIDAKE HEVILTGSDS EISIEITIPK

      70           80           90          100          110          120
TVDGEDIVNI SETGSVVLPG RFFVDIIKKL PGKDVKLSTN EQFQTLITSG HSEFNLSGLD

     130          140          150          160          170          180
PDQYPLLPQV SRDDAIQLSV KVLKNVIAQT NFAVSTSETR PVLTGWNWLI QENELICTAT

     190          200          210          220          230          240
DSHRLAVRKL QLEDVSENKN VIIPGKALAE LNKIMSDNEE DIDIFFASNQ VLFKVGNVNF

     250          260          270          280          290          300
ISRLLEGHYP DTTRLFPENY EIKLSIDNGE FYHAIDRASL LAREGGNNVI KLSTGDDVVE

     310          320          330          340          350          360
LSSTSPEIGT VKEEVDANDV EGGSLKISFN SKYMDALKA IDNDEVEVEF FGTMKPFILK

     370
PKGDDSVTQL ILPIRTY

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P50029 in  
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EMBNET-CH/SIB  
(Switzerland)



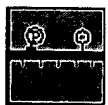
Direct BLAST submission at  
NCBI (Bethesda, USA)



ScanProsite, MotifScan



Sequence analysis tools:  
ProtParam, ProtScale,  
Compute pI/Mw, PeptideMass,  
PeptideCutter, Dotlet (Java)



Feature table viewer (Java)



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L4: Entry 3 of 16

File: USPT

Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287844 B1

TITLE: Compositions and methods for controlling genetically engineered organisms

Brief Summary Text (15):

It is not intended that the present invention be limited to particular polymerases or promoters. In one embodiment, the bacteriophage T7 RNA polymerase is used and the microorganism expresses the polymerase inhibitor, T7 lysozyme.

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L21: Entry 32 of 211

File: USPT

Oct 16, 2001

DOCUMENT-IDENTIFIER: US 6303568 B1

TITLE: Therapeutic antimicrobial polypeptides, their use and methods for preparation

Brief Summary Text (11):

The present invention provides for a method for treatment of an animal having a bacterial infection caused by either or both gram-positive or gram-negative bacteria, such as by a member of the group consisting of *Brucella*, *Listeria*, *Pseudomonas* (other than *P. solanacium*), *Staphylococcus* or a protozoan infection caused by a member of the group consisting of *Trypanosoma* and *Plasmodia*, which method comprises administration to said mammal of an antibacterial amount of an antimicrobial polypeptide selected from the group consisting of a cecropin, an attacin, a lysozyme, a polypeptide transcribed from gene 13 of phage P22, an S protein from lambda phage, and an E protein from phage PhiX174. As another aspect of the present invention, there is provided a biosynthetic method for producing the antimicrobial polypeptides of the present invention which method includes the steps of

**WEST**

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L6: Entry 80 of 120

File: USPT

Sep 22, 1998

DOCUMENT-IDENTIFIER: US 5811654 A

TITLE: Plants genetically enhanced for nutritional quality

Brief Summary Text (37):

The antimicrobial genes in the plants of the present invention generally encode for antibacterial and/or antifungal polypeptides, and/or antiviral agents such as micRNA, not normally found in the particular plant species. Suitable antimicrobial polypeptides are, for example, derived from insect hemolymph, such as attacin. A preferred class of antimicrobial polypeptides include the lytic peptides. Exemplary lytic peptides include lysozymes, cecropins, attacins, melittins, magainins, bombinins, xenopsins, caeruleins, the polypeptide from gene 13 of phage P22, S protein from lambda phage, E protein from phage PhiX174, and the like. However, lytic peptides such as the melittins, bombinins, and magainins are generally relatively high in lytic activity, and are therefore less preferred since host plant cells may be adversely affected thereby.

**WEST**☐  

L6: Entry 119 of 120

File: USPT

Mar 13, 1984

DOCUMENT-IDENTIFIER: US 4436815 A

TITLE: Method for stabilizing and selecting recombinant DNA containing host cells

Brief Summary Text (25):

The interaction of bacteriophage .lambda. with E. coli K12 is employed to illustrate the applicability of cell suicide for maintaining and stabilizing recombinant DNA host cells. Bacteriophage .lambda. is a temperate bacteriophage that follows either of two mutually exclusive cycles when infecting E. coli K12. In the lytic phase the bacteriophage DNA replicates autonomously, directs synthesis and assembly of bacteriophage components, and kills the cells concomitant with the release of mature bacteriophage. In the lysogenic phase the bacteriophage is integrated into the host's chromosome as a prophage, replicates as a marker on the chromosome, and blocks synthesis of bacteriophage components. A bacteriophage gene, .lambda.cI, codes for a repressor that maintains the lysogenic state and blocks expression of genes for bacteriophage components and maturation. If the repressor is inactivated or removed from the cell, the prophage everts from the chromosome, enters the lytic cycle, and kills the cell. Bacteriophage with a defective .lambda.cI gene cannot maintain the lysogenic state and are lethal to the cell unless a functional repressor is provided from an alternate source. In an illustrative embodiment of the present invention, .lambda.cI90 is employed as a repressor dependent prophage and a cI gene, contained in a restriction fragment and cloned into a recombinant DNA cloning vector, serves as the functional repressor.

Brief Summary Text (29):

The cloning of the .about.1.3 kb EcoRI-BamHI trp E-insulin A chain gene containing restriction fragment of plasmid pIA7.DELTA.4.DELTA.1 onto the .about.4.7 kb EcoRI-BamHI restriction fragment of plasmid pPR12, hereinafter designated pPR12.DELTA.2, results in the novel plasmid pPR17. The plasmid pIA7.DELTA.4.DELTA.1 .about.1.3 kb EcoRI-BamHI restriction fragment contains part of .DELTA.2 so therefore the construction restores .DELTA.2 to .DELTA.1. Plasmid pPR17 contains the .about.0.9 kb PstI-HincII restriction fragment of bacteriophage .lambda.cI857 and thus blocks the lytic development of bacteriophage lambda in lysogenized host cells. In addition, plasmid pPR17 codes for and expresses the aforementioned trp E-insulin A chain fused gene product at levels significantly above that of other .lambda.cI gene containing plasmids known in the art. A restriction site and functional map of plasmid pPR17 is presented in FIG. 5 of the accompanying drawings.

Brief Summary Text (32):

The cloning of the .about.1.3 kb EcoRI-BamHI trp E-insulin B chain gene containing restriction fragment of plasmid pIB7.DELTA.4.DELTA.1 onto the .about.4.7 kb EcoRI-BamHI restriction fragment of plasmid pPR12 results in the novel plasmid pPR18. Plasmid pPR18 contains the .about.0.9 kb PstI-HincII restriction fragment of bacteriophage .lambda.cI857 and thus blocks the lytic development of bacteriophage lambda in lysogenized host cells. In addition, plasmid pPR18 codes for and expresses the aforementioned trp E-insulin B chain fused

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## DP3B\_ACTPL (P24701)

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} -  
Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)

## DP3B\_AQUAE (O67725)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR

AQ\_1882} - *Aquifex aeolicus*

DP3B\_BACHD (Q9RCA1)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BH0002}  
- *Bacillus halodurans*

DP3B\_BACSU (P05649)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR DNAG} -  
*Bacillus subtilis*

DP3B\_BORBU (P33761)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BB0438}  
- *Borrelia burgdorferi* (Lyme disease spirochete)

DP3B\_BUCAI (P57127)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BU011} -  
*Buchnera aphidicola* (subsp. *Acyrtosiphon pisum*) (*Acyrtosiphon pisum*  
symbiotic bacterium)

DP3B\_BUCAP (P29439)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BUSG011}  
- *Buchnera aphidicola* (subsp. *Schizaphis graminum*)

DP3B\_BUCRP (Q9EVE4)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - *Buchnera*  
*aphidicola* (subsp. *Rhopalosiphum padi*)

DP3B\_CAUCR (P48198)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR CC0156} -  
*Caulobacter crescentus*

DP3B\_CHLMU (Q9PKW4)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR TC0347}  
- *Chlamydia muridarum*

DP3B\_CHLPN (Q9Z8K0)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR CPN0338  
OR CP0419} - *Chlamydia pneumoniae* (*Chlamydophila pneumoniae*)

DP3B\_CHLTR (O84078)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR CT075} -  
*Chlamydia trachomatis*

DP3B\_ECOLI (P00583)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR B3701 OR  
Z5192 OR ECS4636} - *Escherichia coli*, *Escherichia coli* O157:H7



**DP3B\_HAEIN (P43744)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR HI0992}  
- *Haemophilus influenzae*

**DP3B\_HELPJ (Q9ZLX4)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR JHP0452} - *Helicobacter pylori* J99 (*Campylobacter pylori* J99)

**DP3B\_HELPY (O25242)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR HP0500}  
- *Helicobacter pylori* (*Campylobacter pylori*)

**DP3B\_LACLA (Q9CJJ1)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR LL0002} -  
*Lactococcus lactis* (subsp. *lactis*) (*Streptococcus lactis*)

**DP3B\_LACLC (O54376)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} -  
*Lactococcus lactis* (subsp. *cremoris*) (*Streptococcus cremoris*)

**DP3B\_MICLU (P21174)**

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} -  
*Micrococcus luteus* (*Micrococcus lysodeikticus*)

**DP3B\_MYCBO (O33914)**

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} -  
*Mycobacterium bovis*

**DP3B\_MYCCA (P24117)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} -  
*Mycoplasma capricolum*

**DP3B\_MYCGE (P47247)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR MG001} -  
*Mycoplasma genitalium*

**DP3B\_MYCLE (P46387)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR ML0002}  
- *Mycobacterium leprae*

**DP3B\_MYCPA (Q9L7L6)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} -  
*Mycobacterium paratuberculosis*

**DP3B\_MYCPN (Q50313)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR MPN001}

OR MP153} - *Mycoplasma pneumoniae*

DP3B\_MYCPU (Q98RK6)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR MYPU\_0020} - *Mycoplasma pulmonis*

DP3B\_MYCSM (P52851)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - *Mycobacterium smegmatis*

DP3B\_MYCTU (Q50790)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR RV0002 OR MT0002 OR MTV029.02 OR MTCY10H4.0} - *Mycobacterium tuberculosis*

DP3B\_PROMI (P22838)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - *Proteus mirabilis*

DP3B\_PSEAE (Q9I7C4)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR PA0002} - *Pseudomonas aeruginosa*

DP3B\_PSEPU (P13455)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - *Pseudomonas putida*

DP3B\_RHOCA (P31861)

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - *Rhodobacter capsulatus* (*Rhodopseudomonas capsulata*)

DP3B\_RICCN (Q92I37)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR RC0583} - *Rickettsia conorii*

DP3B\_RICPR (Q9ZDB3)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR RP419} - *Rickettsia prowazekii*

DP3B\_SALTY (P26464)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR STM3837} - *Salmonella typhimurium*

DP3B\_SERMA (P29438)

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - *Serratia marcescens*

## Search in TrEMBL: There are matches to 53 out of 729579 entries

### Q50381

DnaN protein (Fragment) {GENE:DNAN} - *Mycobacterium smegmatis*

### Q8K8Z9

Beta subunit of DNA polymerase III {GENE:DNAN OR SPYM3\_0002} - *Streptococcus pyogenes* (serotype M3)

### Q8KGG7

DNA polymerase III, beta subunit {GENE:DNAN OR CT0001} - *Chlorobium tepidum*

### Q8KQT1

DNA polymerase III beta chain {GENE:DNAN} - *Xanthomonas campestris* (pv. *campestris*)

### Q8P329

Beta subunit of DNA polymerase III {GENE:DNAN OR SPYM18\_0002} - *Streptococcus pyogenes* (serotype M18)

### Q8PEH4

DNA polymerase III beta chain {GENE:DNAN OR XCC0002} - *Xanthomonas campestris* (pv. *campestris*)

### Q8PRG1

DNA polymerase III beta chain {GENE:DNAN OR XAC0002} - *Xanthomonas axonopodis* (pv. *citri*)

### Q8RDL5

DNA polymerase III beta subunit {GENE:DNAN OR TTE0002} - *Thermoanaerobacter tengcongensis*

### Q8RJH9

DNA polymerase III subunit protein (Fragment) {GENE:DNAN-LIKE} - *Streptomyces caespitosus*

### Q8RNR9

DNA polymerase III (Fragment) {GENE:DNAN} - *Streptomyces avermitilis*

### Q8UIJ4

DNA polymerase III, beta chain {GENE:DNAN OR ATU0301 OR AGR\_C\_520} - *Agrobacterium tumefaciens* (strain C58 / ATCC 33970)

Q8XPG1

DNA-directed DNA polymerase III beta chain {GENE:DNAN OR CPE0002} - *Clostridium perfringens*

Q8XTV5

Probable DNA polymerase III (Beta chain) protein (EC 2.7.7.7) {GENE:DNAN OR RSC3441 OR RS01822} - *Ralstonia solanacearum* (*Pseudomonas solanacearum*)

Q8YAW1

DNA polymerase III, beta chain {GENE:DNAN OR LMO0002} - *Listeria monocytogenes*

Q8YVG8

DNA polymerase III beta subunit {GENE:DNAN OR ALR2010} - *Anabaena* sp. (strain PCC 7120)

Q8Z9U8

DNA polymerase III, beta subunit protein (EC 2.7.7.7) {GENE:DNAN OR YPO4096} - *Yersinia pestis*

Q92FV1

DNA polymerase III, beta chain {GENE:DNAN OR LIN0002} - *Listeria innocua*

Q92SN6

Probable DNA polymerase III, beta chain protein (EC 2.7.7.7) {GENE:DNAN OR R00335 OR SMC00415} - *Rhizobium meliloti* (*Sinorhizobium meliloti*)

Q93LM9

DNA polymerase III beta subunit DnaN {GENE:DNAN} - *Cytophaga johnsonae*

Q9A209

Beta subunit of DNA polymerase III (EC 2.7.7.7) {GENE:DNAN OR SPY0003} - *Streptococcus pyogenes*

Q9AD31

Putative DNA-polymerase III, beta chain {GENE:DNAN OR SCP1.119} - *Streptomyces coelicolor* [Plasmid SCP1]

Q9AJ55

DnaN {GENE:DNAN} - *Buchnera aphidicola*

Q9AJ56

DnaN {GENE:DNAN} - Buchnera aphidicola

Q9AJ57

DnaN {GENE:DNAN} - Buchnera aphidicola

Q9AJ58

DnaN {GENE:DNAN} - Buchnera aphidicola

Q9AJ59

DnaN {GENE:DNAN} - Buchnera aphidicola

Q9CLQ5

DnaN {GENE:DNAN OR PM1160} - Pasteurella multocida

Q9EVD9

DNA polymerase III beta subunit (Fragment) {GENE:DNAN} - Rhizobium meliloti (Sinorhizobium meliloti)

Q9EVE6

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVE7

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVE8

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVE9

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF0

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF1

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF2

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF3

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF4

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF5

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF6

DNA polymerase III beta subunit (DnaN) {GENE:DNAN} - Buchnera aphidicola

Q9EVF7

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVF8

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9EVN6

DnaN {GENE:DNAN} - Mycobacterium tuberculosis

Q9EVR1

DNA polymerase III beta subunit (Fragment) {GENE:DNAN} -  
Streptococcus pyogenes

Q9JW44

Putative DNA polymerase III, beta subunit (EC 2.7.7.7) {GENE:DNAN OR  
NMA0553} - Neisseria meningitidis (serogroup A)

Q9PJA9

DNA polymerase III, beta chain (EC 2.7.7.7) {GENE:DNAN OR CJ0002} -  
Campylobacter jejuni

Q9PR66

DNA pol III beta chain {GENE:DNAN OR UU079} - Ureaplasma parvum  
(Ureaplasma urealyticum biotype 1)

Q9REM9

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9REN0

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9REN1

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9REN2

DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola

Q9RNB9

DNA polymerase III beta subunit (EC 2.7.7.7) (Fragment) {GENE:DNAN} -  
Microcystis aeruginosa

Q9X9D6

Putative DnaN protein (Fragment) {GENE:DNAN} - Thermus thermophilus

Q9ZB90

DnaN (Fragment) {GENE:DNAN} - Mycobacterium avium

---

in SWISS-PROT/TrEMBL by AC, ID,  
description, gene name, organism  
**Please do NOT use any boolean  
operators (and, or, etc.)**

---

If you would like to retrieve all the entries contained in this list , you can enter a file name. These entries will then be saved to a file under this name in the directory outgoing of the ExPASy anonymous ftp server, from where you can download it. (Please note that this temporary file will only be kept for 1 week.)

File name:

or

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<a href="#">Hosted by CBR Canada</a>	Mirror sites:	<a href="#">Bolivia</a>	<a href="#">China</a>	<a href="#">Korea</a>	<a href="#">Switzerland</a>	<a href="#">Taiwan</a>	<a href="#">USA</a>



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## HMM Profile Page

Accession #: TIGR00663 Name: dnan

Both TIGRFAMs and Pfams are displayed on this page. TIGRFAMs and Pfams are based on Hidden Markov Models or HMMs. An HMM is a statistical model for a system that can be represented as a succession of transitions between discrete states. Scores are reported both in bits of information and as an E-value. See [here](#) for more information on this TIGRFAM or Pfam and its HMM.

**dnan Information:** See below for detailed information on this family, including cutoff score for inclusion in this family and the average score of genes/proteins in this family. To view all genes with the same EC number, click on the **EC Number** link. To view more information on the Role Category for this family, click on the **Role Category** link.



Accession: TIGR00663

Name: dnan

Is logy equi  
Type:

Common Name: DNA polymerase III, beta subunit

Noise Cutoff: 0.00

Trusted 30.00

Avg. 531.

Cutoff:

Score: 27.0

EC Number: 2.7.7.7

HMM 406

length:

Relationship: InterPro assignment: IPR001001

Role Category: Mainrole: DNA metabolism Subrole: DNA replication, recombination, and r

Gene Ontology (GO) Terms: GO:0003887 function DNA-directed DNA polymer  
GO:0006260 process DNA replication  
GO:0009360 component DNA polymerase III comple

Author(s): Loftus BJ,  
Eisen JA

Created: Dec 8 1999  
12:19PM

Last May  
Modified: 2:32

References: EGAD: 28015  
SWISSPROT: P43744  
SWISSPROT: P00583  
MUID: 84237568 .

Comments: All proteins in this family for which functions are known a  
components of the DNA polymerase III complex (beta sul  
This family is based on the phylogenomic analysis of JA Ei  
(1999, Ph.D. Thesis, Stanford  
University).

Display Hits and Overlaps: To view all CMR proteins that are members of thi  
click on **All CMR Hits**. To display any overlapping HMMs, click on **Any overlap  
HMMs?**

All CMR Hits to TIGR00663

Any Overlapping HMMs?

**Alignment Display:** View a multiple protein alignment display for this HMM. Click on the **FASTA** or **MSF** format and then depress the submit button below. Depress the **JalView** button to start a protein alignment program. This program allows the user to view the alignment in different ways (e.g., highlight the identical and similar amino acids in the alignment).

MSF HMM Alignment ▼

Submit

## Members of the dnan HMM

Listed below are the current members of this HMM, including those not found in CMR. Click on the **Protein ID** link to view the protein report for a particular member of this family.

PROTEIN ID	COORDINATES	DATABASE
CMR: <a href="#">TP0002</a>	1-370	TIGR - CMR
CMR: <a href="#">HI0992</a>	1-366	TIGR - CMR
CMR: <a href="#">NTL01SS00293</a>	1-389	TIGR - CMR
CMR: <a href="#">NTL01BS00002</a>	1-377	TIGR - CMR
CMR: <a href="#">NTL01ML0004</a>	13-397	TIGR - CMR
CMR: <a href="#">BB0438</a>	5-384	TIGR - CMR
SP: <a href="#">P33761</a>	5-384	SWISS-PROT/TrE
CMR: <a href="#">HP0500</a>	1-374	TIGR - CMR
SP: <a href="#">P29439</a>	1-366	SWISS-PROT/TrE

CMR: <u>NTL01HP00448</u>	1-374	TIGR - CMR
CMR: <u>NTL01PM1162</u>	1-366	TIGR - CMR
SP: <u>P34029</u>	1-362	SWISS-PROT/TrE
SP: <u>Q9L7L6</u>	13-397	SWISS-PROT/TrE
CMR: <u>NTL02MT00002</u>	13-400	TIGR - CMR
GP: <u>9845532</u>	13-400	GenBank
GP: <u>11320921</u>	1-366	GenBank
SP: <u>P22838</u>	1-367	SWISS-PROT/TrE
CMR: <u>NTL03EC4650</u>	1-366	TIGR - CMR
SP: <u>P52851</u>	11-395	SWISS-PROT/TrE
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CMR: <u>NTL01ST3835</u>	1-366	TIGR - CMR
GP: <u>15988190</u>	1-366	GenBank
GP: <u>15981986</u>	1-366	GenBank
GP: <u>1321905</u>	13-397	GenBank
CMR: <u>NTL03PA00003</u>	1-367	TIGR - CMR
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CMR: <u>VC0013</u>	1-366	TIGR - CMR
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GP: <u>16409361</u>	1-380	GenBank
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GP: <u>11320919</u>	1-366	GenBank
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GP: <u>11320917</u>	1-367	GenBank
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GP: <u>13398095</u>	1-368	GenBank
GP: <u>11320897</u>	1-368	GenBank
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GP: <u>11320893</u>	1-368	GenBank
GP: <u>17983990</u>	26-397	GenBank
GP: <u>11320895</u>	1-368	GenBank
EGAD: <u>24168</u>	1-374	TIGR - EGAD

CMR: <u>NTL01NM00516</u>	2-367	TIGR - CMR
CMR: <u>SP0002</u>	2-377	TIGR - CMR
CMR: <u>CC0156</u>	1-372	TIGR - CMR
GP: <u>11320901</u>	1-367	GenBank
CMR: <u>NTL02SP0002</u>	2-377	TIGR - CMR
CMR: <u>NMB1902</u>	2-367	TIGR - CMR
CMR: <u>NTL02ML4325</u>	1-372	TIGR - CMR
CMR: <u>NT01MC2948</u>	1-376	TIGR - CMR
GP: <u>17738634</u>	1-372	GenBank
GP: <u>15155199</u>	32-403	GenBank
CMR: <u>NTL01SM00335</u>	1-372	TIGR - CMR
CMR: <u>TM0262</u>	1-365	TIGR - CMR
CMR: <u>NTL01SPL0005</u>	2-377	TIGR - CMR
CMR: <u>TC0347</u>	1-363	TIGR - CMR
CMR: <u>NTL01CT00076</u>	51-413	TIGR - CMR
SP: <u>O84078</u>	51-413	SWISS-PROT/TrE
GP: <u>16885215</u>	2-377	GenBank
CMR: <u>NTL01LL0003</u>	2-379	TIGR - CMR
CMR: <u>CP0419</u>	1-363	TIGR - CMR
CMR: <u>NTL02CP00339</u>	1-363	TIGR - CMR
CMR: <u>NTL01CP00329</u>	1-363	TIGR - CMR
SP: <u>O54376</u>	2-379	SWISS-PROT/TrE
GP: <u>13620601</u>	1-370	GenBank
GP: <u>14531036</u>	1-370	GenBank
CMR: <u>NTL01AA01298</u>	1-363	TIGR - CMR
GP: <u>17134654</u>	1-373	GenBank
GP: <u>15619665</u>	2-379	GenBank

<b>CMR: <u>NTL01CJ00004</u></b>	<b>1-355</b>	<b>TIGR - CMR</b>
<b>CMR: <u>NTL01RP00403</u></b>	<b>2-381</b>	<b>TIGR - CMR</b>
<b>SP: <u>P24117</u></b>	<b>1-373</b>	<b>SWISS-PROT/TrE</b>
<b>CMR: <u>DR0001</u></b>	<b>2-372</b>	<b>TIGR - CMR</b>
<b>GP: <u>7716426</u></b>	<b>1-365</b>	<b>GenBank</b>
<b>SP: <u>P21174</u></b>	<b>1-309</b>	<b>SWISS-PROT/TrE</b>
<b>EGAD: <u>24146</u></b>	<b>1-309</b>	<b>TIGR - EGAD</b>
<b>CMR: <u>PG1853</u></b>	<b>1-374</b>	<b>TIGR - CMR</b>
<b>CMR: <u>NTL01MP00147</u></b>	<b>1-380</b>	<b>TIGR - CMR</b>
<b>CMR: <u>NTL02MP0003</u></b>	<b>1-372</b>	<b>TIGR - CMR</b>
<b>GP: <u>3953450</u></b>	<b>13-258</b>	<b>GenBank</b>
<b>CMR: <u>NTL01UU00079</u></b>	<b>1-379</b>	<b>TIGR - CMR</b>
<b>CMR: <u>MG001</u></b>	<b>1-364</b>	<b>TIGR - CMR</b>
<b>GP: <u>6468432</u></b>	<b>12-384</b>	<b>GenBank</b>

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# InterPro

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## InterPro - Protein P50029

Protein	Match Display
SWISS-PROT DP3B_STAAM P50029	<a href="#">IPR001001</a> <a href="#">PF00712</a> '
	<a href="#">IPR001001</a> <a href="#">PF02767</a> '
	<a href="#">IPR001001</a> <a href="#">PF02768</a>
	<a href="#">IPR001001</a> <a href="#">SM00480</a> —
	<a href="#">IPR001001</a> <a href="#">TIGR00663</a> '

**WEST**

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L6: Entry 6 of 120

File: USPT

Aug 13, 2002

US-PAT-NO: 6432444

DOCUMENT-IDENTIFIER: US 6432444 B1

TITLE: Use of bacterial phage associated lysing enzymes for treating dermatological infections

DATE-ISSUED: August 13, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fischetti; Vincent	West Hempstead	NY		
Loomis; Lawrence	Columbia	MD		

US-CL-CURRENT: 424/443; 424/447, 424/45, 424/450, 424/78.03, 424/78.05, 424/78.06, 424/78.07, 424/94.1, 514/2, 514/937, 514/944, 514/948

## CLAIMS:

What we claim is:

1. A bandage for treating a bacterial infection of skin, wherein said bandage contains a composition produced by the method of: (a) obtaining an effective amount of at least one lytic enzyme genetically coded for by a specific bacteriophage specific for a bacteria infecting said skin, wherein the bacteria to be treated is selected from the group consisting of Staphylococcus, Pseudomonas, Streptococcus, and combinations thereof, wherein said at least one said lytic enzyme is specific for and has the ability to digest a cell wall of one of said bacteria and is coded for by the same said bacteriophage capable of infecting said bacteria being digested; and (b) mixing said at least one lytic enzyme produced in step (a) with a topical carrier selected from the group consisting of an ointment, a cream, an alcohol based liquid, an aqueous liquid, a water soluble gel, a lotion, a non-aqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, protein carriers, a powdered cellulose carmel, and combinations thereof.
2. The composition according to claim 1, further comprising at least one preservative.
3. The composition according to claim 2, wherein said preservative is a bacteriocidal agent or bacteriostatic agent.
4. The composition according to claim 1, further comprising a surfactant in an amount effective to potentiate a therapeutic effect of the composition.
5. The composition according to claim 1, further comprising lystostaphin for the



treatment of *Staphylococcus aureus*.

6. The composition according to claim 1, further comprising a lysozyme.
7. The composition according to claim 1, further comprising at least one antioxidant.
8. The composition according to claim 1, further comprising at least one anti-inflammatory agent.
9. The composition according to claim 1, further comprising an antibiotic.

Entry name	DP3B_STAAM
Primary accession number	P50029
Secondary accession numbers	None
Entered in SWISS-PROT in	Release 34, October 1996
Sequence was last modified in	Release 34, October 1996
Annotations were last modified in	Release 41, June 2002
<b>Name and origin of the protein</b>	
Protein name	DNA polymerase III, beta chain
Synonym	EC <u>2.7.7.7</u>
Gene name	DNAN or <u>SAV0002</u> or <u>SA0002</u> or <u>MW0002</u>
From	<u>Staphylococcus aureus</u> [TaxID: 158878] <u>(strain Mu50 / ATCC 700699)</u> <u>Staphylococcus aureus</u> [TaxID: 158879] <u>(strain N315)</u> <u>Staphylococcus aureus</u> [TaxID: 196620] <u>(strain MW2)</u> <u>Staphylococcus aureus</u> [TaxID: 1280]
Taxonomy	<u>Bacteria</u> ; <u>Firmicutes</u> ; <u>Bacillales</u> ; <u>Staphylococcus</u> .

## References

- [1] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN**=Mu50 / ATCC 700699, and N315;  
**MEDLINE**=21311952; PubMed=11418146; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Kuroda M.](#), [Ohta T.](#), [Uchiyama I.](#), [Baba T.](#), [Yuzawa H.](#), [Kobayashi I.](#), [Cui L.](#), [Oguchi A.](#), [Aoki K.-I.](#), [Nagai Y.](#), [Lian J.-Q.](#), [Ito T.](#), [Kanamori M.](#), [Matsumaru H.](#), [Maruyama A.](#), [Murakami H.](#), [Hosoyama A.](#), [Mizutani-Ui Y.](#), [Takahashi N.K.](#), [Sawano T.](#), [Inoue R.-I.](#), [Kaito C.](#), [Sekimizu K.](#), [Hirakawa H.](#), [Kuhara S.](#), [Goto S.](#), [Yabuzaki J.](#), [Kanehisa M.](#), [Yamashita A.](#), [Oshima K.](#), [Furuya K.](#), [Yoshino C.](#), [Shiba T.](#), [Hattori M.](#), [Ogasawara N.](#), [Hayashi H.](#), [Hiramatsu K.](#);  
 "Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*.";  
 Lancet 357:1225-1240(2001).
- [2] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN**=MW2;  
**MEDLINE**=22040717; PubMed=12044378; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Baba T.](#), [Takeuchi F.](#), [Kuroda M.](#), [Yuzawa H.](#), [Aoki K.-I.](#), [Oguchi A.](#), [Nagai Y.](#), [Iwama N.](#), [Asano K.](#), [Naimi T.](#), [Kuroda H.](#), [Cui L.](#), [Yamamoto K.](#), [Hiramatsu K.](#);  
 "Genome and virulence determinants of high virulence community-acquired MRSA.";  
 Lancet 359:1819-1827(2002).
- [3] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN**=YB886;  
**MEDLINE**=95206242; PubMed=7898435; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Alonso J.C.](#), [Fisher L.M.](#);  
 "Nucleotide sequence of the *recF* gene cluster from *Staphylococcus aureus* and complementation analysis in *Bacillus subtilis recF* mutants.";  
 Mol. Gen. Genet. 246:680-686(1995).

### Comments

**FUNCTION:** DNA POLYMERASE III IS A COMPLEX, MULTICHAIN ENZYME RESPONSIBLE FOR MOST OF THE REPLICATIVE SYNTHESIS IN BACTERIA. THIS DNA POLYMERASE ALSO EXHIBITS 3' TO 5' EXONUCLEASE ACTIVITY. THE BETA CHAIN IS REQUIRED

FOR INITIATION OF REPLICATION ONCE IT IS CLAMPED ONTO DNA, IT SLIDES FREELY (BIDIRECTIONAL AND ATP-INDEPENDENT) ALONG DUPLEX DNA (*BY SIMILARITY*).

**CATALYTIC ACTIVITY:** N deoxynucleoside triphosphate = N diphosphate + {DNA}<sub>N</sub>.

**SUBUNIT:** CONTAINS A CORE (COMPOSED OF ALPHA, EPSILON, AND THETA CHAINS) THAT CAN REPAIR SHORT GAPS CREATED BY NUCLEASE IN DUPLEX DNA. FOR EFFICIENT REPLICATION OF THE LONG, SINGLE-STRANDED TEMPLATES, POL III REQUIRES THE AUXILIARY CHAINS BETA, GAMMA, AND DELTA (*BY SIMILARITY*).

**SUBCELLULAR LOCATION:** Cytoplasmic (*By similarity*).

### Copyright

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### Cross-references

EMBL	AP003358;	[ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ]
	BAB56164.1; -.	[ <a href="#">CoDingSequence</a> ]
	AP003129; BAB41218.1; -.	[ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ]
		[ <a href="#">CoDingSequence</a> ]
	AP004822;	[ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ]
	BAB93867.1; -.	[ <a href="#">CoDingSequence</a> ]
CMR	P50029; SAV0002.	
InterPro	IPR001001; DNA_polIII_beta. <a href="#">Graphical view of domain structure.</a>	
Pfam	PF00712; DNA_pol3_beta; 1.	
	PF02767; DNA_pol3_beta_2; 1.	
	PF02768; DNA_pol3_beta_3; 1.	
SMART	SM00480; POL3Bc; 1.	
TIGRFAMs	TIGR00663; dnan; 1.	
ProDom	[ <a href="#">Domain structure</a> / <a href="#">List of seq. sharing at least 1 domain</a> ].	
BLOCKS	P50029.	
ProtoNet	P50029.	
ProtoMap	P50029.	
PRESAGE	P50029.	
DIP	P50029.	
ModBase	P50029.	
SWISS-2DPAGE	<a href="#">Get region on 2D PAGE.</a>	

## Keywords

[Transferase](#); [DNA-directed DNA polymerase](#); [DNA replication](#);  
[Complete proteome](#).

## Features

None

## Sequence information

Length: 377 AA	Molecular weight: 41913 Da	CRC64: 0A985EF94E044FBC [This is a checksum on the sequence]
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70	80	90	100	110	120
TVDGEDIVNI	SETGSVVLPG	RFFVDIIKKL	PGKDVKLSTN	EQFQTLITSG	HSEFNLSGLD
130	140	150	160	170	180
PDQYPLLPQV	SRDDAIQLSV	KVLKNVIAQT	NFAVSTSETR	PVLTGVNWLI	QENELICTAT
190	200	210	220	230	240
DSHRLAVRKL	QLEDVSENKN	VIIPGKALAE	LNKIMSDNEE	DIDIFFASNQ	VLFKVGNVNF
250	260	270	280	290	300
ISRLLEGHYP	DTTRLFPENY	EIKLSIDNGE	FYHAIDRASL	LAREGGNNVI	KLSTGDDVVE
310	320	330	340	350	360
LSSTSPEIGT	VKEEVDANDV	EGGSLKISFN	SKYMMDALKA	IDNDEVEVEF	FGTMKPFILK
370					
PKGDDSVTQL	ILPIRTY				

P50029 in  
FASTA format

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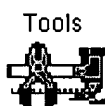
Direct BLAST submission at  
[EMBNET-CH/SIB](#)  
(Switzerland)



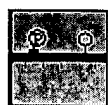
Direct BLAST submission at  
[NCBI \(Bethesda, USA\)](#)



[ScanProsite](#), [MotifScan](#)



Sequence analysis tools:  
[ProtParam](#), [ProtScale](#),  
[Compute pI/Mw](#), [PeptideMass](#),  
[PeptideCutter](#), [Dotlet](#) (Java)



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L14: Entry 1 of 7

File: USPT

Nov 26, 2002

DOCUMENT-IDENTIFIER: US 6485902 B2

TITLE: Use of bacteriophages for control of escherichia coli O157

Other Reference Publication (5):

J. Alisky, K. Iczkowski, A. Rapoport and N. Troitsky, 1998, "Bacteriophages show promise as antimicrobial agents", Journal of Infection 36: 5-15.

Other Reference Publication (13):

Alexander Sulakvelidze, Zemphira Alavidze and J. Glenn Morris Jr., Mar. 2001, "Bacteriophage Therapy", Antimicrobial Agents and Chemotherapy 45: 649-659.



gene product at levels significantly above that of other .lambda.cI gene containing plasmids known in the art. A restriction site and functional map of plasmid pPR18 is presented in FIG. 6 of the accompanying drawings.

**WEST**

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L4: Entry 1 of 7

File: USPT

Aug 13, 2002

DOCUMENT-IDENTIFIER: US 6432444 B1

TITLE: Use of bacterial phage associated lysing enzymes for treating dermatological infections

Other Reference Publication (14):

Loessner, et al. (1999) "Evidence for a holin-like protein gene fully embedded out of frame in the endolysin gene of Staphylococcus aureus Bacteriophage." (1999) Journal of Bacteriology, 181(15) p. 4452-4460.

**WEST**

Generate Collection

Print

L3: Entry 4 of 18

File: USPT

Aug 13, 2002

DOCUMENT-IDENTIFIER: US 6432444 B1

TITLE: Use of bacterial phage associated lysing enzymes for treating dermatological infections

Other Reference Publication (14):

Loessner, et al. (1999) "Evidence for a holin-like protein gene fully embedded out of frame in the endolysin gene of Staphylococcus aureus Bacteriophage." (1999) Journal of Bacteriology, 181(15) p. 4452-4460.

Other Reference Publication (17):

Oki Masaya et al. (1997) "Functional and structural features of the holin HOL protein of the Lactobacillus plantarum phage phi-gle: Analysis in Escherichia coli system." Gene (AMSTERDAM) 197(1-2) p 137-145.

**DP3B\_SPICI (P34029)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} -  
*Spiroplasma citri*

**DP3B\_STAAM (P50029)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SAV0002 OR SA0002 OR MW0002} - *Staphylococcus aureus* (strain Mu50 / ATCC 700699), *Staphylococcus aureus* (strain N315), *Staphylococcus aureus* (strain MW2), *Staphylococcus aureus*

**DP3B\_STRCO (P27903)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SCO3878 OR SCH18.15C} - *Streptomyces coelicolor*

**DP3B\_STRPN (O06672)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SP0002} -  
*Streptococcus pneumoniae*

**DP3B\_SYN7 (P52023)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} -  
*Synechococcus* sp. (strain PCC 7942) (*Anacystis nidulans* R2)

**DP3B\_SYNY3 (P72856)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SLR0965}  
- *Synechocystis* sp. (strain PCC 6803)

**DP3B\_TREPA (O83048)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR TP0002} -  
*Treponema pallidum*

**DP3B\_VIBCH (Q9KVB5)**

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR VC0013} -  
*Vibrio cholerae*

**DP3B\_VIBHA (P52620)**

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} -  
*Vibrio harveyi*

**YRL3\_MYCCA (P43041)**

Hypothetical 20.7 kDa protein in KSGA-DNAN intergenic region (ORF L3).  
- *Mycoplasma capricolum*

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L1: Entry 5 of 16

File: USPT

Apr 24, 2001

DOCUMENT-IDENTIFIER: US 6221642 B1

TITLE: Process for reconstituting the polymerase III\* and other subassemblies of E. coli DNA polymerase III holoenzyme from peptide subunits

Brief Summary Text (8):

Within Pol II, the .alpha. subunit (dnaE) contains the DNA polymerase activity (Blancar et al., 1984, Proc. Natl Acad. Sci. USA, vol. 81, pp 4622-4626), and the .epsilon. subunit (dnaQ, mutD) is the proofreading 3'-5' exonuclease (Scheuermann and Echols, 1985, Proc. Natl Acad. Sci. USA, vol. 81, pp 7747-7751; DeFrancesco et al., 1984, J. Biol. Chem, vol. 259, pp 5567-5573). The .alpha. subunit forms a tight 1:1 complex with .epsilon. (Maki and Kornberg, 1985, J. Biol. Chem., vol. 260, pp 12987-12992). Whereas most DNA polymerases have 3'-5' exonuclease activity, only the holoenzyme relegates this activity to an accessory protein. The following three accessory proteins of the holoenzyme are known to be required for DNA replication as they are products of genes that are essential for cell viability: .beta. (dnaN) (Burgers et al., 1981, Proc. Natl Acad. Sci. USA, vol. 78, pp 5391-5395), .tau., and .gamma. (the latter two both encoded by the dnaXZ gene) (Kodaira et al., 1983, Mol. Gen. Genet., vol. 192, pp 80-86).

Other Reference Publication (18):

Ohmori, H., Kimura, M., Nagata, T., and Sakakibara, Y., "Structural analysis of the dnaA and dnaN genes of Escherichia coli," Gene, 28:159-170 (1984).

**WEST**

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Print

L3: Entry 13 of 18

File: USPT

Jul 10, 2001

DOCUMENT-IDENTIFIER: US 6258560 B1

TITLE: Process for bacterial production of polypeptides

Detailed Description Text (5):

As used herein, "gene t" or "t gene" or "holin" refers to a lytic gene of bacteriophage T4 that is required for lysis but does not appear to have lysozyme activity. See also *Molecular Genetics of Bacteriophage T4*, supra, p. 398-399.

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[\[References\]](#)
[\[Comments\]](#)
[\[Cross-references\]](#)  
[\[Keywords\]](#)
[\[Features\]](#)
[\[Sequence\]](#)
[\[Tools\]](#)

## General information about the entry

Entry name	O80063
Primary accession number	O80063
Secondary accession numbers	None
Entered in TrEMBL in	Release 08, November 1998
Sequence was last modified in	Release 08, November 1998
Annotations were last modified in	Release 23, February 2003

## Name and origin of the protein

Protein name	Holin
Synonyms	None
Gene name	None
From	<u>Staphylococcus aureus</u> [TaxID: <u>bacteriophage PVL</u> 71366]
Taxonomy	<u>Viruses; dsDNA viruses, no RNA stage;</u> <u>Caudovirales; Siphoviridae.</u>

## References

[1]	<p>SEQUENCE FROM NUCLEIC ACID.</p> <p>MEDLINE=98067870; PubMed=9404084; [<a href="#">NCBI</a>, <a href="#">ExPASy</a>, <a href="#">EBI</a>, <a href="#">Israel</a>, <a href="#">Japan</a>]</p> <p><a href="#">Kaneko J.</a>, <a href="#">Muramoto K.</a>, <a href="#">Kamio Y.</a>;</p> <p>"Gene of LukF-PV-like component of Panton-Valentine leukocidin in Staphylococcus aureus P83 is linked with lukM.";</p> <p>Biosci. Biotechnol. Biochem. 61:1960-1962(1997).</p>
[2]	<p>SEQUENCE FROM NUCLEIC ACID.</p> <p>MEDLINE=98332719; PubMed=9666077; [<a href="#">NCBI</a>, <a href="#">ExPASy</a>, <a href="#">EBI</a>, <a href="#">Israel</a>, <a href="#">Japan</a>]</p> <p><a href="#">Kaneko J.</a>, <a href="#">Kimura T.</a>, <a href="#">Narita S.</a>, <a href="#">Tomita T.</a>, <a href="#">Kamio Y.</a>;</p> <p>"Complete nucleotide sequence and molecular characterization of the temperate staphylococcal bacteriophage phi PVL carrying Panton-Valentine leukocidin genes.";</p> <p><a href="#">Gene</a> 215:57-67(1998).</p>

### Comments

None

### Cross-references

EMBL	<p>AB009866; <a href="#">[EMBL / GenBank / DDBJ]</a></p> <p>BAA31897.1; -. <a href="#">[CoDingSequence]</a></p>
InterPro	<p><a href="#">IPR006479</a>; Holin_SPP1.</p> <p><a href="#">Graphical view of domain structure.</a></p>
Pfam	<a href="#">PF04688</a> ; Phage_holin; 1.
TIGRFAMs	<a href="#">TIGR01592</a> ; holin_SPP1; 1.
ProDom	<a href="#">[Domain structure / List of seq. sharing at least 1 domain].</a>
ProtoMap	<a href="#">O80063</a> .
PRESAGE	<a href="#">O80063</a> .
ModBase	<a href="#">O80063</a> .
SWISS-2DPAGE	<a href="#">Get region on 2D PAGE.</a>

### Keywords

None

### Features



None

Sequence information		
Length: 100 AA	Molecular weight: 11175 Da	CRC64: 8DE3F7B4EAB7E595 [This is a checksum on the sequence]
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[NCBI \(Bethesda, USA\)](#)



[ScanProsite](#), [MotifScan](#)



Sequence analysis tools:  
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[Compute pI/Mw](#), [PeptideMass](#),  
[PeptideCutter](#), [Dotlet \(Java\)](#)



Feature table [viewer](#) (Java)



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<a href="#">Hosted by CBR Canada</a>		Mirror sites:		<a href="#">Bolivia</a>	<a href="#">China</a>	<a href="#">Korea</a>	<a href="#">Switzerland</a>	<a href="#">Taiwan</a>	<a href="#">USA</a>

# WEST



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L6: Entry 104 of 120

File: USPT

Aug 22, 1995

DOCUMENT-IDENTIFIER: US 5443969 A

TITLE: RNA packaging system

## Detailed Description Text (41):

In a specific embodiment of the invention detailed in the examples herein, plasmids encoding and capable of expressing TMV CP and capable of transcribing OAS-linked DNA sequences are transformed into a host bacterial strain, referred to as E. coli BL21 (DE3) pLysE that contains a .lambda. lysogen in which the structural gene for bacteriophage T7 RNA polymerase is expressed when the inducer IPTG (isopropyl-beta-D-thiogalactopyranoside) is added to the medium (Studier et al., 1990, Meth. Enzymol. 185:62-89). This particular strain of bacteria also contains the plasmid vector pACYC184 which is a p15A replicon and (as pLysE) carries the T7 lysozyme structural gene. The T7 lysozyme binds to the T7 RNA polymerase and inhibits the basal level of its activity that results from transcription from the lac UV5 promoter even in the absence of the IPTG inducer. As stated supra, the lysozyme also makes the cells more fragile since it degrades the peptidoglycan wall of the bacterial cells. In this particular system, induction of T7 RNA polymerase by addition of IPTG activates expression of OAS-linked DNA under the control of the T7 promoter. A second expression plasmid, which expresses CP under the control of the T7 promoter also commences expression; thus, assembly of OAS-linked RNA molecules into viral particles ensues.

## Detailed Description Text (58):

The host bacterial strain was E. coli BL21 (DE3). E. coli BL21 is F.sup.+, ompT, r.sub..beta..sup.-, m.beta.-. DE3 is a .lambda. derivative which carries a DNA fragment containing the lacI gene, the lac UV5 promoter, the beginning of the lacZ gene, and the gene for T7 RNA polymerase. The bacterial strain was originally provided by the Brookhaven National Research Laboratory and is a .lambda. lysogen in which the structural gene for bacteriophage T7 RNA polymerase is expressed from the lac UV5 promoter when the inducer IPTG is added to the medium (Studier et al., 1990, Meth. Enzymol. 185:62-89). The lysogenic host strain also contains the plasmid vector pACYC184 (Pouwels et al., 1985, in Cloning Vectors, Elsevier Science Publishers, Amsterdam, p. I-A-iv-9) which is a p15A replicon and, as pLysE (Studier et al., 1990, Meth. Enzymol. 185:60-89), carries the T7 lysozyme structural gene expressed from the tet promoter. The lysozyme functions to bind to the T7 RNA polymerase and inhibit the low level of constitutive transcription which occurs from the lac UV5 promoter even in the absence of the IPTG inducer. The lysozyme also makes the cells more fragile since it degrades the peptidoglycan wall of the E. coli cells.

# WEST Search History

DATE: Thursday, January 02, 2003

<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
side by side			result set
	<i>DB=USPT; PLUR=YES; OP=AND</i>		
L1	dna-n or dnan	16	L1
L2	pol.clm.	270	L2
L3	pol\$3.clm.	93558	L3
L4	(\$polymerase or polymerase\$.clm. same (antagon\$ or inhibit\$ or interact\$ or block\$ or inactiv\$ or reduc\$).clm.	273	L4
L5	L4 and (\$phage or phage\$ or bacteriophage or bacteriophages or bacterio-phage).clm.	19	L5
L6	(l2 or l3) and (antagon\$ or inhibit\$ or interact\$ or block\$ or inactiv\$ or reduc\$).clm.	19121	L6
L7	L6 and (\$polymerase or polymerase\$)	337	L7
L8	L7 and (subunit or sub-unit or domain or moiety)	236	L8
L9	(\$phage or phage\$ or bacteriophage or bacteriophages or bacterio-phage)	29432	L9
L10	L9 same (antagon\$ or inhibit\$ or interact\$ or block\$ or inactiv\$ or reduc\$)	9022	L10
L11	L10 same (pol or pol!!! or polymerase or dnan or dna-n or \$polymerase or polymerase\$)	714	L11
L12	L11 and (antibiotic or anti-biotic or antimicrobial or anti-microbial)	354	L12
L13	L11 same(antibiotic or anti-biotic or antimicrobial or anti-microbial)	37	L13

L14	(pol111 or pol3 or pol-111 or pol-3 or pollll or pol-lll)	28	L14
L15	\$polymerase or polymerase\$	29974	L15
L16	L15 same (staphylo\$ or aureus)	220	L16
L17	(antagon\$ or inhibit\$ or interact\$ or block\$ or inactiv\$ or reduc\$)	2268326	L17
L18	L17 same l16	54	L18
L19	L18 and l9	18	L19
L20	L9 near25 (antibiotic or anti-biotic or antimicrobial or anti-microbial or l17)	4270	L20
L21	l20 same (staphylo\$ or aureus or l14 or l15)	211	L21

END OF SEARCH HISTORY

# WEST Search History

DATE: Thursday, January 02, 2003

<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
sid	by side		result set
<i>DB=USPT; PLUR=YES; OP=AND</i>			
L1	antimicrobial or anti-microbial or antibiotic or anti-biotic or antagonist or inhibitor or inhibits or modulator or modulation or blocks or blocker or inactivator or prevents or kills	1922046	L1
L2	L1 near5 polymerase	1629	L2
L3	L2 near10 (subunit or sub or unit or domain or beta)	75	L3
L4	L2 near3 (bacteriophage or bacterio-phage or phage or phage\$)	16	L4
L5	L1 near25 (lysozyme or lytic or lysogenic or lysogenesis)	1193	L5
L6	L5 same (bacteriophage or bacterio-phage or phage or phage\$)	120	L6
L7	(pelletier or gros or debow or de-bow).in. and aureus	8	L7
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=AND</i>			
L8	(pelletier or gros or debow or de-bow).in. and aureus not l7	6	L8
L9	( dubow or du-bow).in. and aureus not l7	6	L9
L10	L9 not l8	0	L10

END OF SEARCH HISTORY

Set	Items	Description
S1	49	'DNA POLYMERASE BETA --ANTAGONISTS AND INHIBITO' OR 'DNA P- OLYMERASE BETA --DRUG EFFECTS --DE'
S2	79	'DNA POLYMERASE BETA --ANTAGONISTS AND INHIBITO'
S3	36	S2 NOT S1
S4	1	'DNA POLYMERASE BETA INHIBITOR'
S5	1	'DNA POLYMERASE III BETA SUBUNIT DNAN GENE'
S6	2	'DNA POLYMERASE III INHIBITOR'
S7	75	E28-E32
S8	1	'DNA POLYMERASE-BETA INHIBITOR'
S9	4	E46-E48
S10	1	'DNA POLYMERIZATION ASSAYS'
S11	1	'DNA POLYMERSE-BETA'
S12	86	S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11
S13	79	RD (unique items)
?t s3/9/15 16		

DIALOG

# IPB001001: DNA\_polIII\_beta

DNA polymerase III, beta chain

- Introduction
- Block number IPB001001A
- Block number IPB001001B
- Block number IPB001001C
- Block number IPB001001D
- Block number IPB001001E
- Block number IPB001001F
- Block number IPB001001G
- Block number IPB001001H
  
- InterPro entry IPR001001 (source of sequences used to make blocks)
  
- Block Map [About Maps]
  
- Logos. [About Logos]  
Select display format: [GIF] [PDF] [Postscript]
  
- Tree from blocks alignment. [About Trees] [About ProWeb TreeViewer]  
[Data] [ProWeb TreeViewer] [XBitmap] [GIF] [PDF] [Postscript]  
[Newick]
  
- PDB entries
  
- Search blocks vs other databases:
  - COBBLER sequence and BLAST searches [About COBBLER]
  - MAST Search of all blocks vs a sequence database [About MAST]
  - LAMA search of all blocks vs a blocks database [About LAMA]

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homologies  
with  
known  
proteins*

- [CODEHOP](#) to design PCR primers from blocks [[About CODEHOP](#)]
- [SIFT](#) to predict amino acid substitutions in blocks [[About SIFT](#)]
- [Additional Links](#)

[\[Return to top\]](#)

## Block IPB001001A

ID	DNA_polIII_beta; BLOCK		
AC	IPB001001A; distance from previous block=(4,54)		
DE	DNA polymerase III, beta chain		
BL	PLL; width=49; seqs=58; 99.5%=2503; strength=1251		
DP3B	BACSU P05649	( 5)	IQKDRLVESVQDVLKAVSSRTTIPILTGIIKIVASDDGVSTGSDSDISI 34
DP3B	BUCAI P57127	( 5)	INNNILIKNLQKISRLLVKNTSLPILDNVLINIKNGMLSLTGTNLEIEL 20
DP3B	CAUCR P48198	( 5)	IERAALLKALGHVQSVVERRNTIPILSNILLSAEGDRLSFSATDLDMEI 31
DP3B	HAEIN P43744	( 5)	ISRENLLKPLQQVCGVLSNRPNIPVLNNVLLQIEDYRLTITGTDLEVEL 30
DP3B	MYCTU Q50790	( 17)	LLRESFADAVSWVAKNLPARPAVPVLSGVLLTGSDNGLTISGFDYEVSA 33
DP3B	PSEPU P13455	( 5)	IQREALLKPLQLVAGVVERRQTLPVLSNVLLVVQQQLSLTGTDLEVEL 21
DP3B	RICPR Q9ZDB3	( 6)	VETKTLMQSLGFARSIIIEKRNVIPEYANIKLSAQDGNLELSSTNMDLYL 79
DP3B	SPICI P34029	( 5)	IKRDKILDELLKVSRIISQKTLIPSLLGILIEVKDKITFTTSDGDTSI 65
DP3B	STAAU P50029	( 6)	IKRDYFITQLNDTLKAISPRTTLPILTGIIKIDAKEHEVILTGSDSEISI 47
DP3B	STRPN O06672	( 6)	INKNFLQLALNITKRAISSKNAIPILSTVKIDVTNEGVTLIGSNGQISI 42
DP3B	SYNY3 P72856	( 5)	CRQSDLSSGLSLVSRAVSSRPTHVPVLGNVLLLEADADKNYLRLTAFDLSL 66
DP3B	ECOLI P00583	( 5)	VEREHLKPLQQVSGPLGGRPTLPILGNLLLQVADGTLSLTGTDLEMEM 25
DP3B	PROMI P22838	( 5)	IEREQLLKPLQQVSGPLGGRPTLPILGNLLLKVTTENTLSLTGTDLEMEM 27
DP3B	SALTY P26464	( 5)	VEREHLKPLQQVSGPLGGRPTLPILGNLLLQVADGTLSLTGTDLEMEM 25
DP3B	AQUAE O67725	( 5)	VDREELEEVLKKARESTEKKAALPILANFLLSAKEENLIVRATDLENYL 71
DP3B	BACHD Q9RCA1	( 5)	IDRDFVQNVNVHVS KAVSSRTTIPILTGIIKIVADHEGVTLTGSDSDISI 34
DP3B	BUCAP P29439	( 5)	IQNDILTKNLKKITRVLVKNISFPILNIIQVEDGTLSLTNTNLEIEL 18
DP3B	CHLMU Q9PKW4	( 5)	ISRNELGNLIKVKQNVVPQSTPIPVLTHVLIESCNDELVFTATDLTVST 43
DP3B	CHLPN Q9Z8K0	( 5)	VSRNELGNLIKVIQSVVPQNTPIPVLTHVLIEYTNDELVFTATDLTVST 40
DP3B	CHLTR O84078	( 55)	ISRNELGNLIKVKQNVVPQSTPIPVLTHVLIESCNDELVFTATDLTVST 43
DP3B	HELPU Q9ZLX4	( 5)	VSKNDLENTLRYLQAFLDKKDASSIASHIHLEVIKEKFLKASDSDIGL 82
DP3B	LACLC O54376	( 6)	INKTAFQNALKITKQAIGSKVTIPALTKLKEVEEKGITLIGSNGQISI 51
DP3B	MYCCA P24117	( 5)	INRIVLLDNLSKAAKVIDYKVNPNPSLSGIYLNVLNDQVNVITTSGLISF 85
DP3B	MYCLE P46387	( 17)	LARESFASAVSWVAKYLPTRPTVPVLSGVLLTGSDSGLTISGFDYEVSA 39
DP3B	MYCPA Q9L7L6	( 17)	LVRESFADAVSWVAKSLPSRPVAVPVLSGVLLSGTDEGLTISGFDYEVSA 30
DP3B	MYCSM P52851	( 15)	VVREDFADAVAWVARSLPTRPTIPVLAGVLLTGTDEGLTISGFDYEVSA 32
DP3B	PSEAE Q9I7C4	( 5)	IQREALLKPLQLVAGVVERRQTLPVLSNVLLVVEGQQQLSLTGTDLEVEL 21



DP3B STRCO	P27903	(	5)	VERDVLAEAVAWAARSLPARPPAPVLAGLLLKAEQGQLSLSSFDYEVSA	40
DP3B SYNPF	P52023	(	5)	CRQNELNTSLSLVSRVPSPRNHPVLANVLLAADAGTQRLSLTAFDLSL	63
DP3B TREPA	O83048	(	5)	CEKEAFLKEISTAQEVISNKKNTSIFSNVLLAAQGALLTIRATDTKVTF	90
Q9RYE8		(	6)	VTKKTNLNEGLGLLERVIPSRSSNPILLTALKVETSEGGTLTSGTNLEIDL	70
Q9REN2		(	5)	IINKIFTQHLKKVNRLLISKNSTLPILNITVNNGIISLTAKNLEIEL	30
Q9REN1		(	5)	IKNKIFIQHLKKVNRLLISKNSTLPILNITVNNGIISLTATNLETEL	18
Q9REN0		(	5)	IKNKIFTQHLKKINRLITKNSTLPILNITVNNGIISLTATNLETEL	18
Q9REM9		(	5)	IKNKIFTQHLKKINRLISKNTLPILNITVNNDIVSLTATNLETEL	18
Q9PJA9		(	5)	INKNTLESAILCNAYVEKKDSSTITSHLFFHADEKLLIKASDYEIGI	100
Q9PHE2		(	5)	LQRETFLKPLAHVVNVVERRQTRSILANLLIKVNEDQLSLTGTDLLEVEL	38
Q9KVX5		(	5)	IERSHLIKPLQQVSGTLGGRASLPILGNLLKVEENQLSMTATDLEVEL	35
Q9JXS8		(	6)	AERDSSLKPLQAVTGIVERRHTLPILSNVLEIEGKGGQTKLLATDLEIQI	37
Q9JW44		(	6)	AERDSSLKPLQAVTGIVERRHTLPILSNVLEIEGKGGQTKLLATDLEIQI	37
Q9EVN6		(	17)	LLRESFADAVSWAKNLPARPAVPVLSGVLLTGSDNGLTISGFDYEVSA	33
Q9EVF8		(	5)	INNKFIFQNLQKINRFITKNISFPILNITVNNGIISLTATNLEIEL	14
Q9EVF7		(	5)	INNKFIFQNLQKINRFITKNISFPILNITVNNGIISLTATNLEIEL	14
Q9EVF5		(	5)	INNKFIFQNLQKINRFITKNISFPILNITVNNGIISLTATNLEIEL	13
Q9EVF4		(	5)	INNKFIFQNLQKINRLITKNISFPILNITVNNGIISLTATNLEIEL	13
Q9EVF3		(	5)	INNKFIFQNLQKINRFITKNISFPILNITVNNGIISLTATNLEIEL	17
Q9EVF2		(	5)	INNKFIFQNLQKINRFITKNISFPILNITVNNGIISLTATNLEIEL	16
Q9EVF1		(	5)	IKNSIFIKNLQKINRFVKNISFPILNITVNNGIISLTATNLEIEL	16
Q9EVF0		(	5)	IKNNIFVQHLQKVNRFITKNISFPILNITVNNGIISLTATNLEIEL	14
Q9EVE9		(	5)	IKNNIFVQHLQKVNRFITKNISFPILNITVNNGIISLTATNLEIEL	21
Q9EVE8		(	5)	INNKFIFIQYLKKVNRFITKNISFPILNITVNNGIISLTATNLEIEL	16
Q9EVE7		(	5)	IKNKIFIQNLQKINRFITKNISFPILNITVNNGIISLTATNLEIEL	13
Q9EVE6		(	5)	IKNNILIHNLQKINRFVTKNNTFPILNITVNNGIISLTATNLEIEL	16
Q9EVE5		(	5)	INNINILIKNLQKISRLLVKNISFPILNITVNNGIISLTATNLEIEL	20
Q9EVE4		(	5)	IQNDILVENLKKITRLLIKNVSPILNITVNNGIISLTATNLEIEL	23
Q9CLQ5		(	5)	VSRENLLKPLQQVCGVLSSRPNIPVLNNVLLQIRGERLVITGTDLEVEL	30
Q9CJJ1		(	6)	INKNAFQNALRITKQAIGSKVTIPALTKLIEVEENGITLIGSNGQISI	45
Q9EVF6		(	5)	INNKFIFTQNLQKINRFITKNISFPILNITVNNGIISLTATNLEIEL	15

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[\[Return to top\]](#)

## Block IPB001001B

ID DNA\_polIII\_beta; BLOCK  
AC IPB001001B; distance from previous block=(10,19)  
DE DNA polymerase III, beta chain  
BL GDI; width=15; seqs=58; 99.5%=930; strength=1097  
DP3B BACSU|P05649 ( 73) GSIVLQARFFSEIVK 28

DP3B BUCAI|P57127 ( 66) GTATISGRKLLDICR 15

DP3B CAUCR|P48198 ( 65) GQITAPAHTLYEIVR 45

DP3B HAEIN|P43744 ( 66) GTFTIPAKKFLDICR 16

DP3B PSEPU|P13455 ( 66) GEITVPARKLMDICK 16

DP3B RICPR|Q9ZDB3 ( 66) GEITVATQTLSDIVR 42

DP3B SPICI|P34029 ( 68) GSVLIKKNKFIVEVIR 46

DP3B STAAU|P50029 ( 74) GSVVLPGRFFVDIIK 24

DP3B STRPN|O06672 ( 73) GSILLEASFFINVVS 47

DP3B SYNY3|P72856 ( 67) GRITLPAKLLNDIVS 22

DP3B ECOLI|P00583 ( 66) GATTVPARKFFDICR 12

DP3B PROMI	P22838	( 66)	GATTVPARKFFDIWR	25
DP3B SALTY	P26464	( 66)	GATTVPARKFFDICR	12
DP3B MYCTU	Q50790	( 77)	GSVLVSGRLLSDITR	15
DP3B AQUAE	Q67725	( 65)	GEVCVHSQKLYDIVK	46
DP3B BACHD	Q9RCA1	( 73)	GSIVLQAKVFAEIVK	40
DP3B BUCAP	P29439	( 66)	GKTTISGRKILNICR	17
DP3B CHLMU	Q9PKW4	( 65)	GSVTIPSRFFQLIR	28
DP3B CHLPN	Q9Z8K0	( 65)	GAISIPSKRFFQLVK	50
DP3B CHLTR	O84078	( 115)	GSVTIPSRFFQLIR	28
DP3B HELPJ	Q9ZLX4	( 66)	GVGTINGKKFLDIIS	41
DP3B LACLC	O54376	( 73)	GSVLLEAAFFENVVS	36
DP3B MYCCA	P24117	( 70)	GKVLKPKYVLEMLR	100
DP3B MYCLE	P46387	( 77)	GSVLVSGRLLSDITR	15
DP3B MYCPA	Q9L7L6	( 77)	GSVLVSGRLLSDIVR	12
DP3B MYCSM	P52851	( 75)	GSVLVSGRLLSDITK	17
DP3B PSEAE	Q9I7C4	( 66)	GEITVPARKLMDICK	16
DP3B STRCO	P27903	( 65)	GTVLVSGRLLADISR	27
DP3B SYNPF	P52023	( 67)	GAITLPAKLLNDIVS	20
DP3B TREPA	O83048	( 65)	GTTTVFCDKLVNVVS	58
Q9RYE8		( 66)	ENFVVPAPHLFAQIVR	64
Q9REN2		( 66)	GSITVSGQKLLNICQ	23
Q9REN1		( 66)	GSITVSGQKLLNICR	11
Q9REN0		( 66)	GSITVSGQKLLNICR	11
Q9REM9		( 66)	GSITVSGQKLLNIXQ	22
Q9PJA9		( 66)	GFATANAKSIADVIK	57
Q9PHE2		( 65)	GEITIPARKIYEIVR	20
Q9KVX5		( 66)	GSITVPARKFLDICR	9
Q9JXS8		( 66)	FRITTNAKKFQDILR	47
Q9JW44		( 66)	FRITTNAKKFQDILR	47
Q9EVN6		( 77)	GTVLVSGRLLSDITR	16
Q9EVF8		( 66)	GNITVSSRKLLDICR	9
Q9EVF7		( 66)	GNTTVSSRKLLDICR	10
Q9EVF5		( 66)	GNTTVSSRKLLDICR	10
Q9EVF4		( 66)	GNTTVSSRKLLDICR	10
Q9EVF3		( 66)	GNTTVSSQKLLDICR	11
Q9EVF2		( 66)	GHITVSSRKILDICR	19
Q9EVF1		( 66)	GSTTVSSRKLLDICR	9
Q9EVF0		( 66)	GSITVSSRKLLQICR	12
Q9EVE9		( 66)	GSITVSSRKLLQICR	12
Q9EVE8		( 66)	GSTTVSSQKILDICR	13
Q9EVE7		( 66)	GSTTVSGRKLLDICR	9
Q9EVE6		( 66)	GSTTISSRKLLDICR	10
Q9EVE5		( 66)	GTATISGRKLLDICR	15
Q9EVE4		( 66)	GKITISGRKILNICR	16
Q9CLQ5		( 66)	GSFTIPAKKFLDICR	15
Q9CJJ1		( 73)	GSVLLEAAFFENVVS	36
Q9EVF6		( 66)	GNTTVSSRKLLDICR	10

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[\[Return to top\]](#)

## Block IPB001001C

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ID  DNA_polIII_beta; BLOCK
AC  IPB001001C; distance from previous block=(16,24)
DE  DNA polymerase III, beta chain
BL  FLP; width=19; seqs=58; 99.5%=1181; strength=1136
DP3B BACSU|P05649 ( 106) IRSGKAEFNLNGLDADEYP 25
DP3B BUCAI|P57127 ( 99) IISGNSRYILTTLPYDSFP 22

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DP3B CAUCR	P48198	( 100)	IQAGRSRFLNPLVLPAGDFP	45
DP3B HAEIN	P43744	( 99)	VQSGRSRFTLATQPAEEYP	36
DP3B MYCTU	Q50790	( 109)	LTCGNARFSLPTMPVEDYP	23
DP3B RICPR	Q9ZDB3	( 99)	IKGQNCKFNLFTLPVSSFP	70
DP3B SPICI	P34029	( 101)	IKANNFDSVLNTLNSADYP	75
DP3B STAAU	P50029	( 107)	ITSGHSEFNLSGLDPDQYP	45
DP3B SYNY3	P72856	( 106)	ITSESGRFQIRGLDADDFP	46
DP3B ECOLI	P00583	( 99)	VRSGRSRFSLSLTPAADFP	18
DP3B PROMI	P22838	( 99)	VRSGRSRFSLSLTPASDFP	21
DP3B PSEPU	P13455	( 99)	VKAGRSRFTLSTLPANDFP	20
DP3B SALTY	P26464	( 99)	VRSGRSRFSLSLTPAADFP	18
DP3B STRPN	O06672	( 106)	LTSGKSEITLKGKDSEQYP	50
DP3B AQUAE	O67725	( 97)	ITGGKSTYKLPTAPAEDFP	43
DP3B BACHD	Q9RCA1	( 106)	IRSGSSVFNLNGLDPDEYP	37
DP3B BUCAP	P29439	( 99)	ISSENSNYILSTLSADTFP	34
DP3B CHLMU	Q9PKW4	( 98)	ITSGSSCFRLLSMGKEDFP	40
DP3B CHLPN	Q9Z8K0	( 98)	ITSGSSCFRLLSMEKEDFP	47
DP3B CHLTR	O84078	( 148)	ITSGSSCFRLLSMGKEDFP	40
DP3B HELPJ	Q9ZLX4	( 98)	IKQNKSSFKLPMFDADEF	47
DP3B LACLC	O54376	( 106)	LTSGKSEITLKGDLSEIYP	37
DP3B MYCCA	P24117	( 103)	IKTNNSDFSIGVLNSEDYP	72
DP3B MYCLE	P46387	( 109)	LTCGSARFSLPTMAVEDYP	28
DP3B MYCPA	Q9L7L6	( 109)	LNCGSARFSLPTMAVEDYP	38
DP3B MYCSM	P52851	( 107)	LTCGSARFSLPTLAVEDYP	26
DP3B PSEAE	Q9I7C4	( 99)	VKAGRSRFTLSTLPANDFP	20
DP3B STRCO	P27903	( 97)	VVCGSSRFTLHTLPVEEYP	33
DP3B SYNPF	P52023	( 100)	LSVGSGQYQMRGISADEFP	91
DP3B TREPA	O83048	( 99)	PPNKKISFQLRTLSEHFP	100
Q9RYE8		( 98)	VRSGGSDFKLQTDIEAYP	68
Q9REN2		( 99)	IISDNSNYILTTLPSDNFP	16
Q9REN1		( 99)	IASDNSNYILTTLPSENF	26
Q9REN0		( 99)	ISLENSNYILNTLPAENFP	31
Q9REM9		( 99)	IVSDDSNYILTTLPSDNFP	33
Q9PJA9		( 98)	VRQKSTKYKLPMFNHEDFP	74
Q9PHE2		( 98)	LQAGRSRFTLATLPANDFP	25
Q9KVX5		( 99)	VRSGRSRFSLATLPASDFP	22
Q9JXS8		( 99)	LKAGKSRFALQTLPAADFP	26
Q9JW44		( 99)	LKAGKSRFALQTLPAADFP	26
Q9EVN6		( 109)	LTCGNARFSLPTMPVEDYP	23
Q9EVF8		( 99)	IISDTSQYILRTLPAENFP	16
Q9EVF7		( 99)	IISDTSQYILRTLPAENFP	16
Q9EVF5		( 99)	IISNTSQYILRTLPAENFP	18
Q9EVF4		( 99)	IISNTSQYILRTLPAENFP	18
Q9EVF3		( 99)	IISDTSQYILRTLPAENFP	16
Q9EVF2		( 99)	IISDKSQYILTTLPADNFP	15
Q9EVF1		( 99)	IISDKSHYILTTLPADNFP	16
Q9EVF0		( 99)	VISDKSHYILNTLPAENFP	18
Q9EVE9		( 99)	VISEKSHYILNTLPSDDFP	20
Q9EVE8		( 99)	ITSDNSHYILKTLPIDNFP	23
Q9EVE7		( 99)	IISDKSHYVLTTLPAENFP	21
Q9EVE6		( 99)	IISDKSKYILTTLPADNFP	23
Q9EVE5		( 99)	IISGNSRYILTTLPYDSFP	22
Q9EVE4		( 99)	VSCENSNYILSTLSADDFP	24
Q9CLQ5		( 99)	VKSGRSKFNLSTLPAAEYP	21
Q9CJJ1		( 106)	LTSGKSEITLKGDLSEIYP	37

Q9EVF6 ( 99) IISDTSQYILRTLPA DNFP 16  
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[\[Return to top\]](#)

## Block IPB001001D

ID DNA\_polIII\_beta; BLOCK  
 AC IPB001001D; distance from previous block=(15,23)  
 DE DNA polymerase III, beta chain  
 BL FRG; width=24; seqs=58; 99.5%=1415; strength=1171  
 DP3B BUCAI |P57127 ( 135) LKKMIEKIQFSMAKQDVRYYLNGI 8  
 DP3B CAUCR |P48198 ( 136) LIRLIDKTRFAISTEETRYYLNGL 15  
 DP3B MYCTU |Q50790 ( 144) FAEAISQVAIAAGRDDTLPMLTGI 14  
 DP3B RICPR |Q9ZDB3 ( 135) FAKIIESTKFSISLDETRYNLNGI 20  
 DP3B SPICI |P34029 ( 137) LKEIISQTSFAIGEKEKRIVFNGL 19  
 DP3B STRPN |O06672 ( 142) LKKIINETAFAASTQESRPILTGV 13  
 DP3B SYNY3 |P72856 ( 142) LNEGLRGALFAASTDETKQVLTGV 34  
 DP3B BACSU |P05649 ( 142) LKNLIRQTVFAVSTSETRPILTGV 13  
 DP3B STAAU |P50029 ( 143) LKNVIAQTNFAVSTSETRPVLTGV 21  
 DP3B ECOLI |P00583 ( 135) MKRLIEATQFSMAHQDVRYYLNGM 12  
 DP3B HAEIN |P43744 ( 135) LRRLIEATQFSMANQDARYFLNGM 11  
 DP3B PROMI |P22838 ( 135) LKRLIESTQFSMAHQDVRYYLNGM 8  
 DP3B PSEPU |P13455 ( 135) LRRLIERTSFAMAQQDVRYYLNGM 8  
 DP3B SALTY |P26464 ( 135) MKRLIESTQFSMAHQDVRYYLNGM 12  
 DP3B AQUAE |O67725 ( 132) LVNGIEKVEYAIKEEANIALQGM 43  
 DP3B BACHD |Q9RCA1 ( 142) LKDIIRQTVFAVSTQETRPVLTGV 10  
 DP3B BUCAP |P29439 ( 135) LKEMIEKTEFSMGKQDVRYYLNGM 6  
 DP3B CHLMU |Q9PKW4 ( 134) LKDMFQRTSFASVREESRYVLTGV 13  
 DP3B CHLPN |Q9Z8K0 ( 134) LKTMLQRTSFASVREESRYVLTGV 13  
 DP3B CHLTR |O84078 ( 184) LKDMFQRTSFASVREESRYVLTGV 13  
 DP3B HELPJ |Q9ZLX4 ( 141) IAPVIEQTSHKRELAGVLMQFNQK 100  
 DP3B LACLC |O54376 ( 142) LKEIFTETVFAVSTQENRPIFTGV 18  
 DP3B MYCCA |P24117 ( 139) VKKTIYQVFSMNENNKKLILTGL 52  
 DP3B MYCLE |P46387 ( 144) FAEAIGQVAIAAGRDTLPMLTGI 20  
 DP3B MYCPA |Q9L7L6 ( 144) FAEAIGQVAIAAGRDDTLPMLTGI 14  
 DP3B MYCSM |P52851 ( 142) FAEAIGQVAVAAGRDDTLPMLTGI 16  
 DP3B PSEAE |Q9I7C4 ( 135) LRRLIDRTSFAMAQQDVRYYLNGM 10  
 DP3B STRCO |P27903 ( 132) FASAVQQVAIAAGRDDTLPVLTGV 25  
 DP3B SYNPF |P52023 ( 136) LIEGLRGTLFATSGDETKQILTGV 31  
 DP3B TREPA |O83048 ( 135) LRNMINTHTVFAVSEDSTRHFINGV 42  
 Q9RYE8 ( 133) LSRAFSSVRYAASNEAFQAVFRGI 53  
 Q9REN2 ( 135) LKKMIEKTQFSMGKQDVRYYLNGI 6  
 Q9REN1 ( 135) LKKMIEKTQFSMGKQDVRYYLNGM 5  
 Q9REN0 ( 135) LKKMIEKTHFSMGKQDVRYYLNGM 5  
 Q9REM9 ( 135) LKKMIEKTQFSMGKQDVRYYLNGI 6  
 Q9PJA9 ( 134) LSRSLKKILPSIDTNNPKYSLNGA 58  
 Q9PHE2 ( 134) LKELIERTAFAMAQQDVRYYLNGL 9  
 Q9KVX5 ( 135) LRGLIEKTQFSMANQDVRYYLNGM 11  
 Q9JXS8 ( 136) FKTMLSQVQYSMAVQDIRYYLNGL 16  
 Q9JW44 ( 136) FKTMLSQVQYSMAVQDIRYYLNGL 16  
 Q9EVN6 ( 144) FAEAISQVAIAAGRDDTLPMLTGI 14  
 Q9EVF8 ( 135) LKEMIEKTHFSMGKQDVRYYLNGM 5

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Q9EVF7      ( 135) LKSMIEKTHFSMGKQDVRYYLNGM    7
Q9EVF5      ( 135) LKDMIEKTHFSMGKQDVRYYLNGM    6
Q9EVF4      ( 135) LKEMIEKTHFSMGKQDVRYYLNGM    5
Q9EVF3      ( 135) IKEMIEKTHFSMGKQDVRYYLNGM    8
Q9EVF2      ( 135) LKEMIEKTHFSMGKQDVRYYLNGM    5
Q9EVF1      ( 135) LKTMIEKTHFSMGKQDVRYYLNGM    6
Q9EVF0      ( 135) LREIEKIYFSMGKQDVRYYLNGM   11
Q9EVE9      ( 135) LKEMIEKTHFSMGKQDVRYYLNGM    5
Q9EVE8      ( 135) LRDMIEKTHFSMGKQDVRYYLNGM    9
Q9EVE7      ( 135) LKEMIEKTQFSMGKQDVRYYLNGM    5
Q9EVE6      ( 135) IREMIKTHFSMGKQDVRYYLNGM    8
Q9EVE5      ( 135) LKKMIEKIQFSMGKQDVPIYLNIGI   16
Q9EVE4      ( 135) LKEMIEKTEFSMGKQDVRYYLNGM    6
Q9CLQ5      ( 135) LRRLIEATQFSMANQDARYFLNGM   11
Q9CJJ1      ( 142) LKEIFTETVFAVSTQENRPIFTGV   18
Q9EVF6      ( 135) LKEMIEKTHFSMGKQDVRYYLNGM    5
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[\[Return to top\]](#)

## Block IPB001001E

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ID  DNA polIII beta; BLOCK
AC  IPB001001E; distance from previous block=(4,14)
DE  DNA polymerase III, beta chain
BL  TDR; width=11; seqs=58; 99.5%=710; strength=1161
DP3B SPICI|P34029 ( 173) ITATDSFRLSC 42

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DP3B BACSU|P05649 ( 176) CTATDSHRLAL 22
DP3B BUCAI|P57127 ( 169) AVATDGYRLGI 12
DP3B CAUCR|P48198 ( 174) AVATDGHRLAL 13
DP3B ECOLI|P00583 ( 169) TVATDGHRLAV 12
DP3B HAEIN|P43744 ( 169) TVATDGHRLAV 12
DP3B MYCTU|Q50790 ( 178) LAATDRFRLAV 21
DP3B PROMI|P22838 ( 169) TVATDGHRLAV 12
DP3B PSEPU|P13455 ( 169) AVSTDGHRLAL 19
DP3B RICPR|Q9ZDB3 ( 169) AASTDGYRLSI 24
DP3B SALT|P26464 ( 169) TVATDGHRLAV 12
DP3B STAAU|P50029 ( 177) CTATDSHRLAV 21
DP3B STRPN|O06672 ( 177) TVATDSHRLSQ 21
DP3B SYNY3|P72856 ( 176) FAATDGHRLAV 15

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DP3B AQUAE|O67725 ( 166) FVGS DGHRLAL 47
DP3B BACHD|Q9RCA1 ( 176) CTATDSHRLAM 23
DP3B BUCAP|P29439 ( 169) SVATDGYRLAI 13
DP3B CHLMU|Q9PKW4 ( 168) VVGTDGKRLAK 25
DP3B CHLPN|Q9Z8K0 ( 168) IVGTDGKRLAK 26
DP3B CHLTR|O84078 ( 218) VVGTDGKRLAK 25
DP3B HELPJ|Q9ZLX4 ( 170) VVGTDTKRLSY 41
DP3B LACLC|O54376 ( 177) AVATDSHRMSQ 28
DP3B MYCCA|P24117 ( 173) FSTTDSFRISQ 100
DP3B MYCLE|P46387 ( 178) LAATDRFRLAV 21
DP3B MYCPA|Q9L7L6 ( 178) LAATDRFRLAV 21
DP3B MYCSM|P52851 ( 176) LAATDRFRLAV 21
DP3B PSEAE|Q9I7C4 ( 169) SVATDGHRLAM 15
DP3B STRCO|P27903 ( 166) LASTDRYRFAV 48
DP3B SYNPF|P52023 ( 170) FAATDGHRLAV 15
DP3B TREPA|O83048 ( 169) CVSTDGKRLAY 25
Q9RYE8      ( 167) VVASDGYRVAI 65
Q9REN2      ( 169) AVATDGYRLGI 12
Q9REN1      ( 169) AVATDGYRLGI 12
Q9REN0      ( 169) TVATDGYRLGI 13

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<u>Q9REM9</u>	( 169)	AVATDGYRLGI	12
<u>Q9PJA9</u>	( 168)	FVGTDTKRLAI	34
<u>Q9PHE2</u>	( 168)	CVATDGHRLAL	14
<u>Q9KVX5</u>	( 169)	SVATDGHMAV	21
<u>Q9JXS8</u>	( 170)	LVATDGHRLAY	14
<u>Q9JW44</u>	( 170)	LVATDGHRLAY	14
<u>Q9EVN6</u>	( 178)	LAATDRFRLAV	21
<u>Q9EVF8</u>	( 169)	MVATDGYRLGM	14
<u>Q9EVF7</u>	( 169)	MVATAGYRLGM	72
<u>Q9EVF5</u>	( 169)	MVATDGYRLGI	12
<u>Q9EVF4</u>	( 169)	MVATDGYRLSI	14
<u>Q9EVF3</u>	( 169)	MVATDGYRLGI	12
<u>Q9EVF2</u>	( 169)	MVATDGYRLGI	12
<u>Q9EVF1</u>	( 169)	MVATDGYRLGI	12
<u>Q9EVF0</u>	( 169)	MVATDGYRLGI	12
<u>Q9EVE9</u>	( 169)	IVATDGYRLGT	28
<u>Q9EVE8</u>	( 169)	MVATDGYRLAL	12
<u>Q9EVE7</u>	( 169)	MVATDGYRLAI	11
<u>Q9EVE6</u>	( 169)	MIATDGYRLGI	35
<u>Q9EVE5</u>	( 169)	AVATDGYRLGI	12
<u>Q9EVE4</u>	( 169)	SVATDGYRLAI	13
<u>Q9CLQ5</u>	( 169)	TVATDGHRLAV	12
<u>Q9CJJ1</u>	( 177)	AVATDSHRMSQ	28
<u>Q9EVF6</u>	( 169)	MVATDGYRLGM	14
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[\[Return to top\]](#)

## Block IPB001001F

ID DNA\_polIII\_beta; BLOCK  
AC IPB001001F; distance from previous block=(9,25)  
DE DNA polymerase III, beta chain  
BL VPE; width=11; seqs=58; 99.5%=720; strength=1034  
DP3B RICPR|Q9ZDB3 ( 193) VILPQKSAEEI 51

DP3B SPICI|P34029 ( 196) VIIPGKFINEI 42

DP3B STRPN|O06672 ( 201) VVIPSRSLREF 33

DP3B SYN3|P72856 ( 212) VTIPARALREL 31

DP3B BACSU|P05649 ( 201) VVIPGKSLTEL 21  
DP3B STAAU|P50029 ( 201) VIIPGKALAEI 17

DP3B BUCAI|P57127 ( 193) IVIPRKGVIEL 13  
DP3B CAUCR|P48198 ( 199) VIVPRKTIAEA 17  
DP3B ECOLI|P00583 ( 193) VIVPRKGVIEL 11  
DP3B HAEIN|P43744 ( 193) VILPRKGVLEL 14  
DP3B MYCTU|Q50790 ( 204) VLVPAKTLAEA 23  
DP3B PROMI|P22838 ( 193) VIVPRKGVIEL 11  
DP3B PSEPU|P13455 ( 194) VIVPRKGILEL 11  
DP3B SALTY|P26464 ( 193) VIVPRKGVIEL 11

DP3B AQUAE|O67725 ( 187) LLIPRKSILKVL 66  
DP3B BACHD|Q9RCA1 ( 203) VVIPGKSLNEL 21  
DP3B BUCAP|P29439 ( 193) IIPNKAVMEL 29  
DP3B CHLMU|Q9PKW4 ( 193) YIIPKAVEEI 28  
DP3B CHLPN|Q9Z8K0 ( 193) YIIPKAVEEI 28  
DP3B CHLTR|O84078 ( 243) YIIPKAVEEI 28  
DP3B HELPJ|Q9ZLX4 ( 197) CILPKRALLEI 51  
DP3B LACLC|O54376 ( 201) VILPSKSINSF 40

DP3B MYCCA	P24117	( 198)	ITIPFKTALEL	53
DP3B MYCLE	P46387	( 204)	VLVPAKTLVEV	39
DP3B MYCPA	Q9L7L6	( 204)	VLVPAKTLAEA	23
DP3B MYCSM	P52851	( 202)	VLVPAKTLAEA	23
DP3B PSEAE	Q9I7C4	( 194)	VIVPRKGILEL	11
DP3B STRCO	P27903	( 192)	ALVPAKTLQDT	70
DP3B SYNPF	P52023	( 196)	VTVPSTRALRDL	48
DP3B TREPA	O83048	( 194)	VIVPTKILGIV	100
Q9RYE8		( 189)	LIIPARSVDEL	40
Q9REN2		( 193)	AIIPRRGIIEI	21
Q9REN1		( 193)	VIIPRKGIIEL	10
Q9REN0		( 193)	AIIPRKGIIEL	16
Q9REM9		( 193)	VIIPRRGIIEI	15
Q9PJA9		( 191)	FSIPKKAIMEM	91
Q9PHE2		( 193)	IILPRKGVMEI	16
Q9KVX5		( 193)	IIVPRKGVLEL	12
Q9JXS8		( 194)	VILPRKTVLEL	15
Q9JW44		( 194)	VILPRKTVLEL	15
Q9EVN6		( 204)	VLVPAKTLAEA	23
Q9EVF8		( 195)	IIMTRKGIIEL	18
Q9EVF7		( 195)	IIMTRKGIIEL	18
Q9EVF5		( 194)	IVMTRKGIIEL	21
Q9EVF4		( 194)	IIMTRKGIIEL	18
Q9EVF3		( 194)	IIMTRKGIIEL	18
Q9EVF2		( 194)	IVMTRRGIIEL	26
Q9EVF1		( 194)	IIIARKGIIEI	21
Q9EVF0		( 195)	IIIARKGITEL	23
Q9EVE9		( 195)	IIIARKGITEL	23
Q9EVE8		( 194)	IVIARQGIIEI	47
Q9EVE7		( 195)	IVITRQGIIEI	41
Q9EVE6		( 194)	IIIITRKGITEL	17
Q9EVE5		( 193)	IVIPRKGVIEL	13
Q9EVE4		( 193)	IIIPSKAVMEI	17
Q9CLQ5		( 193)	VILPRKGVLEL	14
Q9CJJ1		( 201)	VILPSKSINSF	40
Q9EVF6		( 195)	IIMTRKGIIEL	18
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[\[Return to top\]](#)

## Block IPB001001G

ID DNA\_polIII\_beta; BLOCK  
AC IPB001001G; distance from previous block=(21,36)  
DE DNA polymerase III, beta chain  
BL SGP; width=16; seqs=58; 99.5%=1004; strength=1179  
DP3B BUCAI|P57127 ( 234) TQLIEGQYPDYKSVLL 18

DP3B MYCTU|Q50790 ( 251) TRLLDAEFKFRQLLP 30

DP3B RICPR|Q9ZDB3 ( 238) SKLIDGTFPDYSAFIP 50

DP3B SPICI|P34029 ( 228) QKIIEGKYPDTSKVIR 83

DP3B SYN3|P72856 ( 255) SRKLEGAYPAYDQLIP 68

DP3B BACSU|P05649 ( 242) SRLLDGNYPDTTSLIP 27  
DP3B STAAU|P50029 ( 242) SRLLEGHYPDTTRLFP 34  
DP3B STRPN|O06672 ( 242) TRLLEGNYPDTRLIP 25

DP3B CAUCR|P48198 ( 240) SKVIDGAFPDYMVRIP 32  
DP3B ECOLI|P00583 ( 234) SKLVDGRFPDYRRVLP 21

DP3B HAEIN	P43744	( 234)	SKLIDGRFPDYRRVLP	16
DP3B PROMI	P22838	( 235)	SKLVDGRFPDYRRVLP	21
DP3B PSEPU	P13455	( 235)	SKLVDGKFPDYERVLP	20
DP3B SALT	P26464	( 234)	SKLVDGRFPDYRRVLP	21
DP3B AQUAE	O67725	( 229)	VRLLEGEFPDYMSVIP	24
DP3B BACHD	Q9RCA1	( 244)	SRLLEGKYPVTKNMIP	72
DP3B BUCAP	P29439	( 234)	TQLIEGEYPDYKSVLF	22
DP3B CHLMU	Q9PKW4	( 233)	TKLLSGEFPDFSPVIS	36
DP3B CHLPN	Q9Z8K0	( 233)	TKLLSGEFPDFSPVIS	36
DP3B CHLTR	O84078	( 283)	TKLLSGEFPDFSPVIS	36
DP3B HELPJ	Q9ZLX4	( 235)	TKLIDGNYPDYQKILP	29
DP3B LACLC	O54376	( 242)	SRLIEGSYPDTNRLIP	24
DP3B MYCCA	P24117	( 238)	SNLIDGKFPNVQIAFP	100
DP3B MYCLE	P46387	( 251)	TRLLDAEFKPKFRQLLP	30
DP3B MYCPA	Q9L7L6	( 251)	TRLLDAEFKPKFRQLLP	30
DP3B MYCSM	P52851	( 249)	TRLLDAEFKPKFRQLLP	30
DP3B PSEAE	Q9I7C4	( 235)	SKLVDGKFPDYERVLP	20
DP3B STRCO	P27903	( 237)	TRLLEGDLPKYKTLFP	73
DP3B SYNPF	P52023	( 239)	SRTLGDGQYPNYGQLIP	54
DP3B TREPA	O83048	( 234)	SVLIEGQFPNYKRVIP	39
Q9RYE8		( 228)	LKLLDGDGFPDYERVIP	39
Q9REN2		( 234)	VQLIEGEYPDYKSVLL	19
Q9REN1		( 234)	VQLIEGEYPDYKSILL	21
Q9REN0		( 234)	VQLIEGEYPDYKSILS	25
Q9REM9		( 234)	VQLIEGEYPDYKSILL	21
Q9PJA9		( 229)	TKLINDKFPDYEKVIP	83
Q9PHE2		( 234)	SKLIDGSFPDYEGVIP	23
Q9KVX5		( 234)	SKLVDGRFPDYRRVLP	21
Q9JXS8		( 235)	SKVIDGKFPDFNRPVIP	25
Q9JW44		( 235)	SKVIDGKFPDFNRPVIP	25
Q9EVN6		( 251)	TRLLDAEFKPKFRQLLP	30
Q9EVF8		( 236)	AQLIEGKFPNYDSLFL	18
Q9EVF7		( 236)	AQLIEGKFPNYDSLFL	18
Q9EVF5		( 235)	AQLIEGKFPNYNSIFL	20
Q9EVF4		( 235)	AQLIEGIFPNYDSLFL	28
Q9EVF3		( 235)	AQLIEGKFPNYDSVFL	18
Q9EVF2		( 235)	AQLIEGKFPNYDSLFL	18
Q9EVF1		( 235)	AQLIEGKFPNYDSLFL	37
Q9EVF0		( 236)	AQLIEGKFPDYESIFL	18
Q9EVE9		( 236)	AQLIEGKFPNYESIFL	19
Q9EVE8		( 235)	AQLIEGTFPNYTSVFL	25
Q9EVE7		( 236)	AQLIEGKFPNYESIFL	19
Q9EVE6		( 235)	AQLIEGQFPNYESVLF	23
Q9EVE5		( 234)	TQLIEGQYPDYKSVLL	18
Q9EVE4		( 234)	TQLIEGEYPDYESVLF	22
Q9CLQ5		( 234)	SKLIDGRFPDYRRVLP	16
Q9CJJ1		( 242)	SRLIEGSYPDTNRLIP	24
Q9EVF6		( 236)	AQLIEGKFPNYNGLFL	24

[\[Return to top\]](#)

## Block IPB001001H

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ID   DNA_polIII_beta; BLOCK
AC   IPB001001H; distance from previous block=(58,71)
DE   DNA polymerase III, beta chain
BL   GYL; width=20; seqs=58; 99.5%=1114; strength=1193
DP3B BACSU|P05649 ( 323) GEELNISFSPKYMLDALKVL 40

DP3B BUCAI|P57127 ( 312) GNTVKISINVYIIEILNSI 19

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DP3B CAUCR	P48198	( 318)	GEPFEIGFNARYLLDVCQI	47
DP3B HAEIN	P43744	( 312)	GEELEVGFNVITYILDVLNAL	15
DP3B MYCTU	Q50790	( 328)	GEPLTIAFNPTYLTDGLSSL	15
DP3B PSEPU	P13455	( 313)	GSSLEIGFNVSYLLDVLGVM	27
DP3B RICPR	Q9ZDB3	( 325)	DESLVIGFNPQYLEDVLKAI	35
DP3B SPICI	P34029	( 308)	GTDQIIAFNSKYILDALKAF	37
DP3B STAAU	P50029	( 322)	GGSLKISFNSKYMDALKAI	21
DP3B STRPN	O06672	( 322)	GEDLTISFNPTYLIDSLKAL	21
DP3B SYNY3	P72856	( 335)	GEGGQIAFNIKYLMDGLKAL	32
DP3B ECOLI	P00583	( 312)	GAEMEIGFNVSYVLDVLNAL	21
DP3B PROMI	P22838	( 313)	GEEMEIGFNVSYLLDVLNTL	13
DP3B SALTY	P26464	( 312)	GTEMEIGFNVSYVLDVLNAL	17
DP3B AQUAE	O67725	( 307)	GEPFEIGFNGKYLMEALDAY	39
DP3B BACHD	Q9RCA1	( 325)	GEELRISFNGKNVIDALKVV	43
DP3B BUCAP	P29439	( 312)	GEKIEISINVYYLLDVINNI	23
DP3B CHLMU	Q9PKW4	( 311)	GETLEIAFNPFFFLDILKHS	23
DP3B CHLPN	Q9Z8K0	( 311)	GELLEIAFNPFFFLDILKHS	28
DP3B CHLTR	O84078	( 361)	GETLEIAFNPFFFLDILKHS	23
DP3B HELPJ	Q9ZLX4	( 313)	EKAFLGVNAKFFLEALNAL	64
DP3B LACLC	O54376	( 324)	GNDLSISFNPEYLIDALKVI	16
DP3B MYCCA	P24117	( 319)	NKSLSISFNTRFLIDAIKTL	41
DP3B MYCLE	P46387	( 328)	GEPLTIAFNPNYLTDLASV	27
DP3B MYCPA	Q9L7L6	( 328)	GEPLTIAFNPTYLTDGLGSV	18
DP3B MYCSM	P52851	( 326)	GEPLTIAFNPTYLTDGLGSL	15
DP3B PSEAE	Q9I7C4	( 313)	GGNLEIGFNVSYLLDVLGVI	15
DP3B STRCO	P27903	( 314)	GDDISIAFNPTFLDGLSAI	17
DP3B SYNPF	P52023	( 319)	GEPLTIAFNRYLAEGLKAL	26
DP3B TREPA	O83048	( 316)	GESEVIALNYLYLEEPLKVF	74
Q9RYE8		( 308)	EQAMSLAFNARHVLDALGPI	59
Q9REN2		( 312)	GDDIEISINVYYIIEVLNVI	13
Q9REN1		( 312)	GDDIEISINVYYIIEVLSVI	15
Q9REN0		( 312)	GENIEISINVYYIIEVLNVI	15
Q9REM9		( 312)	GDDIEISINVYYIIEVLNVI	13
Q9PJA9		( 304)	SEEFNLTIKIKHLLDFTSI	100
Q9PHE2		( 312)	VDGLAIGFNVNYLLDALSSL	32
Q9KVS5		( 312)	GEPIEIGFNVSYILDVLNTL	12
Q9JXS8		( 313)	GGELEVGFNIGYLMVDLRNI	28
Q9JW44		( 313)	GGELEVGFNIGYLMVDLRNI	28
Q9EVN6		( 328)	GEPLTIAFNPTYLTDGLSSL	15
Q9EVF8		( 314)	GPSIKISINVYYILDILNSI	9
Q9EVF7		( 314)	GPSIKISINVYYILDILNSI	9
Q9EVF5		( 313)	GPSIKISINVYYILDVLNSI	9
Q9EVF4		( 313)	GPSIKISINVYYILDVLNSI	9
Q9EVF3		( 313)	GPSIKISINVYYMLDILNSI	13
Q9EVF2		( 313)	GPSIKISINVYYILDILNSI	9
Q9EVF1		( 313)	GPSIKISINVYYILDILNAI	9
Q9EVF0		( 314)	GPSIEISINVYYILDILNVI	9
Q9EVE9		( 314)	GPSIEISINVYYILDILNVI	9
Q9EVE8		( 313)	NPSIEISINVYYILDILNTI	16
Q9EVE7		( 314)	GPEIEISINVYYILDILNAI	9
Q9EVE6		( 313)	GPVIEISINVYYILDILNSI	15
Q9EVE5		( 312)	GNTVKISINVYYIIEILNSI	19
Q9EVE4		( 312)	GDTIEISINVYYLLDVINNI	19
Q9CLQ5		( 312)	GEEMEIGFNVSYILDVLNAL	17

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Q9CJJ1      ( 324) GNDLAISFNPEYLIDALKVI  18
Q9EVF6      ( 314) GPSIKISINVYYILDILNSI   9
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[\[Return to top\]](#)

## COBBLER sequence (region containing Blocks only)

To do a BLAST search, copy the cobbler sequence below then click on a BLAST link

[\[Blast Search\]](#) [\[Gap-Blast Search\]](#) [\[PSI-Blast Search\]](#)

COBBLER sequence:

```
>IPB001001 DP3B_PROMI|P22838 from 1 to 342 with embedded consensus blocks
mkfiINRNTLLES LQKVS RVISKRTTIPILSNILIEVENDQLTLTGTDLEISLmarvslsqsheiGSVTVPARKFLDIVR
glpegaeisvelgdgdrllITSGNSRFTLRTLPAEDFPnlddwqseveftlpqatLKEMIEQTQFAMSKQDTRYLNGVlf
etentelrMVATDGYRLAVcamdigqslpghsVIIPRKGIMELmrllldgsgesllqlqigsnnlrahvgdfiftSKLIDG
KFPDYERVIPknptktviagcdilkqafsraailsnekgfrgvrinltngqlkitannpegeaeaeivdvqyqGEELEISF
NVKYL LDV LNAIkceevkl11t
```

[\[Return to top\]](#)

## Additional Links (separate browser window)

[MetaFam IPB001001](#)

IPB001001A : [CYRCA IPB001001A](#)

[\[Blocks home\]](#)

**WEST**

Generate Collection

Print

L1: Entry 12 of 16

File: USPT

Mar 18, 1997

DOCUMENT-IDENTIFIER: US 5612182 A

TITLE: Mycobacteriophage specific for the mycobacterium tuberculosis complex

Detailed Description Text (29):

A second potential open reading frame ORF (ORF 1 of NheI-D) was aligned with the DNA polymerase III .beta.-subunit of *Streptomyces coelicolor*. The polymerase III .beta. subunit is the product of the *S. coelicolor* dnaN gene. The alignment showed significant homology of 35% over 360 amino acids. It is likely that translation of ORF 1 (NheI-D) begins at the valine GTG initiator at nucleotide 390. Use of these sequences for translation allows good alignment of both the amino and C-terminal portions of the proteins. ORF 1 (NheI-D) also shows weaker homology to the analogous proteins from *E. coli* and *B. subtilis*, probably as a result of the closer phylogenetic relationship between mycobacteria and streptomyces than between mycobacteria and *E. coli* or *B. subtilis*. However, class III-type DNA polymerases were previously unknown in phage. Phage polymerases are either of type I (Taq, klenow, L5 phage, T coliphages) or of type II (phi29). The type III enzymes are multisubunit enzymes previously found only in bacteria where they are known to be involved in DNA replication and repair. The beta subunit is not known to catalyze DNA replication by itself, but instead appears to play a role as a DNA clamp which provides processivity. Thus, if ORF 1 (NheI-D) is a bona fide DNA polymerase subunit, the other subunits might reside in the DS6A genome, or be supplied by the host cell. The highly processive nature of class III DNA polymerases makes them desirable for use in vitro in nucleic acid amplification and DNA syntheses, etc. ORF 1 (NheI-D) of DS6A may therefore be cloned and expressed in transformed host cells to produce a new recombinant class III DNA polymerase useful in these methods.

**BEST AVAILABLE COPY**

**WEST**

Generate Collection

Print

L1: Entry 10 of 16

File: USPT

May 27, 1997

DOCUMENT-IDENTIFIER: US 5633159 A

TITLE: DNA polymerase III .beta.-subunit from mycobacteriophage DS6A

Detailed Description Text (29):

A second potential open reading frame ORF (ORF 1 of NheI-D) was aligned with the DNA polymerase III .beta.-subunit of *Streptomyces coelicolor*. The polymerase III .beta. subunit is the product of the *S coelicolor dnaN* gene. The alignment showed significant homology of 35% over 360 amino acids. It is likely that translation of ORF 1 (NheI-D) begins at the valine GTG initiator at nucleotide 390. Use of these sequences for translation allows good alignment of both the amino and C-terminal portions of the proteins. ORF 1 (NheI-D) also shows weaker homology to the analogous proteins from *E. coli* and *B. subtilis*, probably as a result of the closer phylogenetic relationship between mycobacteria and streptomyces than between mycobacteria and *E. coli* or *B. subtilis*. However, class III-type DNA polymerases were previously unknown in phage. Phage polymerases are either of type I (Taq, klenow, L5 phage, T coliphages) or of type II (phi29). The type III enzymes are multisubunit enzymes previously found only in bacteria where they are known to be involved in DNA replication and repair. The beta subunit is not known to catalyze DNA replication by itself, but instead appears to play a role as a DNA clamp which provides processivity. Thus, if ORF 1 (NheI-D) is a bona fide DNA polymerase subunit, the other subunits might reside in the DS6A genome, or be supplied by the host cell. The highly processive nature of class III DNA polymerases makes them desirable for use in vitro in nucleic acid amplification and DNA syntheses, etc. ORF 1 (NheI-D) of DS6A may therefore be cloned and expressed in transformed host cells to produce a new recombinant class III DNA polymerase .beta.-sub unit useful in these methods.

**BEST AVAILABLE COPY**